A STUDY OF VARIOUS FACTORS INFLUENCING SELECTED CONSTITUENTS OF BLOOD, URINE AND FAECAL FLUIDS OF PONIES.

JANET M. NICHOLSON.

DOCTOR OF PHILOSOPHY UNIVERSITY OF EDINBURGH 1973

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DECLARATION

In compliance with Edinburgh University Regulation 2.4.15 I, the undersigned, declare that this thesis, composed by myself, describes work which is my own.

Janet M. Nicholson

SUMMARY OF THE THESIS

Selected constituents of the blood, urine and faecal fluids of Shetland and Shetland-cross ponies were studied and considerable variations between and within individual ponies were observed. Wide ranges of values were observed in the untreated ponies, and thus it is likely that erroneous judgements could result from attempts to assess normality or abnormality on the basis of a single observation.

The packed cell volume percentages of these ponies were similar to those reported in other Shetland-type ponies and draught horses. Factors affecting the packed cell volume percentage both *in vivo* and *in vitro* are discussed. The plasma sodium concentration values of the ponies were lower than those found in other equines.

The daily water loss in urine was approximately equal to the water lost in faeces. Of the urinary and faecal fluid constituents studied only inorganic phosphate was lost in greater quantities in the faecal fluid than in the urine. The estimated daily intake of sodium, potassium and chloride was compared with daily losses in urine and faecal fluid, and in all cases daily losses in urine and faecal fluids were less than the quantities ingested.

The concentrations of selected blood constituents were monitored hourly for eight hours before the ponies were presented with their daily hay ration, and for eight hours after they commenced feeding. After feeding began

increases in the packed cell volume percentage and in the concentrations in plasma of urea and sodium were observed. Some decreases in plasma chloride and inorganic phosphate concentrations occurred. Thus it was evident that standardisation of the time of blood sampling in relation to feeding time was essential when the effect of an experimental treatment upon these blood constituents was to be studied.

The plasma and "thiocyanate space" volumes were measured by the dilution of T-1824 and sodium thiocyanate respectively, and the approximate total quantities of certain plasma constituents were estimated. The oral administration of 7.5 litres (35 - 46 ml/kg body weight) of water caused increases in the plasma volume of 1.4% to 8.6%, and in the "thiocyanate space" volume of 7.0% to 21.6%. Fluctuations in the packed cell volume percentage and the concentration of selected plasma constituents did not reflect the magnitude and time of plasma volume expansion indicated by T-1824. Changes in the volume and composition of the urine voided after water loading are described and discussed.

The ingestion of ammonium chloride induced a metabolic acidosis with concurrent respiratory compensation. The clinical signs of metabolic acidosis were evident. Urinary pH decreased and a change in urinary excretion of net base to net acid took place. The ponies were able to excrete net acid even when the pH of the urine was greater than that of the blood. Ammonium chloride ingestion induced

natriuresis without diuresis or hyponatraemia. Other changes in the blood and urine constituents outwith the normal ranges are described and discussed.

The administration of sodium bicarbonate in a dose equivalent to that of ammonium chloride induced few changes in blood acid/base parameters. Although urinary net base excretion increased markedly no significant increases in urine pH were detected, and hence net acid/base excretion was believed to be a more accurate indication of the acid base status of the pony and the renal response to acid/base disturbances than the pH of the urine. Since both ammonium chloride and sodium bicarbonate induced natriuresis without diuresis it was apparent that this phenomenon could arise regardless of whether the source of sodium was exogenous or endogenous. A possible relationship between urinary sodium and inorganic phosphate excretion is discussed. Changes outwith the norm of other blood and urine constituents are also described and discussed.

GENERAL INTRODUCTION

The various blood constituents of man, both in health and in disease, have been extensively studied. In the course of investigating the physiological and biochemical regulatory mechanisms involved in the maintainance of the normal state, and in the studies of the clinical conditions associated with disturbances of the blood constituents, dogs and small laboratory mammals have proved invaluable experimental subjects. Consequently considerable information on these species has been amassed. It was logical that the food producing species would also receive attention, but considering the large increase in the popularity and monetary value of equines over the last decade, investigations of this nature on horses and ponies, and the application of such information to their clinical problems are surprisingly few.

Bearing in mind the horse's evolution, the concept of "fright and flight" and its ingestion of large amounts of base and potassium by virtue of its herbivorous diet, and also man's selection of the species for strenuous work, often under adverse conditions, it would not be surprising if equines differed from other mammals in some aspects of their physiology. The work herein was undertaken first to define the concentration ranges of selected blood constituents in a small group of Shetland and Shetland-cross ponies and then to study factors capable of disturbing these constituents. The relative importance of urinary and faecal routes of excretion of various constituents of plasma studied was also investigated.

In that context, having shown that diurnal variations occurred in the concentrations of some plasma constituents, a study was then made of the effect of feeding on these.

Under some natural circumstances the horse has irregular access to water, and therefore needs to imbibe large volumes at infrequent intervals. Thus, the effect of rapid ingestion of a volume of water approximating to 4% of body weight on plasma and thiocyanate space volumes, and consequently on plasma electrolyte and urea concentrations was examined. The renal response to water loading was also examined concurrently.

Normal ranges of blood pH, pCO_2 and plasma or serum bicarbonate concentrations in man are well established, and the clinical implications of acid/base disturbances have received much attention. However, although equine acid/base parameters have been measured, no ranges of normal values have been widely accepted. Therefore the acid/base status of untreated ponies was examined before attempts were made to disturb their normal state by administering acidifying and alkalosing agents. Electrolyte disturbances commonly accompanying acidosis and alkalosis in man and domestic animals (chiefly the dog) have been reported, but no reference to such disturbances in equines was discovered. Because of their possible clinical implications, changes in the concentrations of selected plasma constituents were studied concurrently with acid/base disturbances. Changes in urine pH and in the urinary electrolyte, urea, and net acid/base content were also investigated simultaneously, so the renal responses to acid/base disturbances of the ponies could be compared with those of other species.

SECTION No.1

THE COLLECTION AND ANALYSIS OF BLOOD, URINE AND FAECAL FLUID FROM UNTREATED PONIES. VALUES OF SELECTED BLOOD, URINE AND FAECAL FLUID PARAMETERS.

PART 1

BLOOD

INTRODUCTION

(i) VENOUS PACKED CELL VOLUME PERCENTAGE

Many authors have determined the venous haematocrit of horses and ponies[1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17]. Much work has been undertaken in an effort to assess the value of a knowledge of this parameter, and to attempt to elucidate the extent to which it represents "true" or "whole body" haematocrit[9 18]. A review of published results is presented in Table No.1.

From Table No.1 a general trend towards higher packed cell volumes in thoroughbred horses is evident[4 9 13], though unfortunately some authors did not specify the breed of the animals from which they derived their results.

Schalm[13] included Arabs as well as Thoroughbreds in his definition of "hot-blooded" horses, and it was his opinion that the normal range of the venous haematocrit of hot-blooded horses had higher limits than the equivalent range for cold-blooded animals.

Marcilese, Valsecchi, Figueiras, Camberos and Varela[9] found a trend towards a higher haematocrit in thoroughbreds, and a lower one in saddle horses and draught animals. They found these differences significant ($p<0.05$). Furthermore, they discovered that only in the first few months of life, and in advanced years, was age likely to markedly influence the venous haematocrit.

TABLE NO.1

THE PACKED CELL VOLUME PERCENTAGES OF EQUINE BLOOD SAMPLES CITED IN PUBLISHED WORK

Reference	$\bar{x}$ % PCV	S.D. or S.E.	Range	No. Observations	Type of Animal
1	28	-	24-34	36	Clydesdales
	30	-	26-40	26	Clydesdales, Vanners, Hunters, Ponies.
			28-33	2	Shetland Ponies
2	35	-	-	1	Working Gelding
3	38.7	4.4 S.D.	30-49	110	Thoroughbreds, Standardbreds, Quarter Horses, Polo Ponies, Hacks.
4	40.9	1.15 S.E.	-	18	Thoroughbreds
	36.5	0.95 S.E.	-	24	Non-Thoroughbreds
	38.4	0.77 S.E.	-	42	Thoroughbreds and Non-Thoroughbreds.
5	33.4				
6	33	-	-	-	Adult Horse
7	-	-	32-44	12	Horses, Ponies, Donkey, Jennet.

TABLE No. 1 (Contd.)

Reference	$\bar{x}$ % PCV	S.D. or S.E.	Range	No. Observations	Type of Animal
8			40.3-42.0	132	Thoroughbreds
9	42.7	4.77 S.D.	32-52	31	Racehorses (T.B.)
	37.3	3.43 S.D.	32-42	6	Saddle Horses.
	33.5	2.87 S.D.	30-37	14	Percheron X Draught Horses
10	43.3	-	37-56	-	Light Horses
	40.3	-	37-46	-	Heavy Horses
11	-	-	31-55	156	Thoroughbreds
12	39	-	32-43	8	7 Clydesdales, 1 Thoroughbred
13	42	-	32-55	-	Thoroughbreds and Arabs
	35	-	24-44	-	Cold Blooded Horses
14	48.3	0.82 S.E.	-	34	Racehorses (T.B.)
	44.8	0.26 S.E.	-	34	Thoroughbreds
	35.3	0.98 S.E.	-	8	) Thoroughbred and Halfbred
	35.7	1.16 S.E.	-	8	) Hacks
15	38.96	-	-	57	Standardbreds
	36.95	-	-	42	Standardbreds
16	34.5	-	29.5-36.3	5	Donkeys
17	27.8	4.19 S.D.	22.4-35.7	11	Ponies
		1.32 S.E.			

Marcilese *et al.*[9] furthered their investigations of haematocrit values. By using ^{51}Cr and ^{59}Fe to label erythrocyte globin and plasma β globulin respectively, independent measurements of erythrocyte and plasma volumes were made, and blood volume was given as the sum of these two measurements. From these data the so-called "body" haematocrit was calculated, and was found in all cases to be lower than venous haematocrit as measured by Wintrobe's method[19] by about 4%. Similar findings have been reported by other workers[18 20 21] although Deavers, Rosborough, Garner, Huggins and Amend[17] discovered the converse situation in their ponies.

Tasker[3] reported no very marked differences in the venous packed cell volume percentages of the breeds he investigated. However, no draught horses were included in his study. Tasker[3] proposed a normal range of 32-52% for equine venous haematocrit values, and he did not differentiate between different breeds or types of animal. Littlejohn[4] found a statistically significant difference ($p<0.05$) between the packed cell volumes of the 18 thoroughbreds and the 24 non-thoroughbreds (the latter consisting mainly of ponies and riding-school horses) from which he obtained blood samples.

Dalton[12] included one thoroughbred in a group of eight horses whose venous packed cell volumes he measured, but which result was derived from the thoroughbred is not stated. Stewart and Holman[1] included two Shetland ponies

in their group of 26 equines of various breeds and types. Deavers, Rosborough, Garner, Huggins and Amend[17] investigated eleven Shetland type animals of both sexes whose ages ranged from 10 months to 4 years, and whose weights ranged from 68-147kg. Their results are similar to those from two Shetland ponies studied by Stewart and Holman[1].

Two other authors[8 11] investigated the possibility of there being a correlation between the age of a horse and its venous haematocrit. Collins[11] noted that the packed cell volume percentages of jugular blood samples from his horses were higher in animals of three years and over than in those below this age. Archer's[8] results from thoroughbreds whose ages ranged from birth to maturity fell within remarkably narrow limits, and he found no correlation between age and haematocrit in his horses.

(ii) ELECTROLYTES AND UREA

The serum and plasma electrolytes of a variety of types and species of equines have been investigated.

Published values of sodium concentrations in equine serum and plasma are summarised in Table No. 2. No evidence suggested that the sodium concentrations in plasma and in serum differ.

Bernstein's[22] results were markedly higher than those of others. Alexander's[23] mean value was the lowest published, and it will be discussed later. After studying both his own results and those obtained by other workers Tasker[3] proposed a normal range of equine plasma sodium

concentrations of 132-146 mEq/l. He included a large number of breeds and types of horses and ponies in this range.

Tevik, Nelson and Lumb[24] obtained a set of results from the same group of horses on each of two separate occasions. Their results did not show a statistically significant difference, though the presentation of their data may have concealed day-to-day variations within individual horses.

Littlejohn[4] reported that plasma sodium concentrations did not differ significantly between the thoroughbred and non-thoroughbred horses he examined. His results were higher than those of Sreter[14].

A summary of the equine serum and plasma potassium concentrations found by various workers is presented in Table No.3. Tasker's[3] lowest result of 1.7 mEq/l appears outstandingly low, though the limited amount of information supplied by some other authors could conceal the discovery of similar levels by them. Tasker[3 26] proposed that a range of 2.4 to 4.7 mEq potassium/l plasma should be considered normal for the horse. Bernstein[22] discovered much higher plasma potassium levels than were reported by others.

It is evident from the data listed in Table No.3 that, excluding Bernstein's[22] results, there was a general trend towards the concentration of potassium in serum being higher than in plasma. This observation will be discussed at a later stage.

Determinations of chloride concentrations in equine

TABLE No.2

SODIUM CONCENTRATIONS IN EQUINE SERUM AND PLASMA CITED IN PUBLISHED WORK

Reference	$\bar{x}$ Na(mEq/l)	S.D. or S.E.	Range	No. Observations	Type of Animal
1	138^{s}	-	133-148	34	Clydesdales
	135^{s}	-	131-137	15	Clydesdales, Vanners, Hunters, Shetland Ponies
5	147.2^{s}			20	Adult Stallions
23	132^{s}	0.5 S.E.		13	Shetland & Shetland-cross Ponies.
3	139^{p}	3.5 S.D.	134-147	101	Thoroughbreds, Standardbreds, Quarter Horses, Polo Ponies.
4	141^{p}	0.63 S.E.	-	40	Thoroughbreds, Non-Thoroughbreds
6	135^{p}				Adult Horse
14	136.6^{p}	0.58 S.E.			Race Horses (T.B.)
	136.8^{p}	0.83 S.E.			Thoroughbreds.
16	136^{p}		130-139	5	Donkeys
22			$152-156^{p}$		
24	139.6^{p}	3.23 S.D.	-	12	Adult Horses
	140.3^{p}	2.42 S.D.	-	12	Adult Horses.

s = serum p = plasma

TABLE No.3

POTASSIUM CONCENTRATIONS IN EQUINE SERUM AND PLASMA CITED IN THE LITERATURE

Reference	$\bar{x}$ K(mEq/l)	S.D. or S.E.	Range	No. Observed	Type of Animal
1	4.22^{s}	-	2.99-7.01	34	Clydesdales
	4.68^{s}	-	3.32-7.03	25	Clydesdales, Vanners, Hunters, Ponies.
	-	-	4.09-4.42	2	Shetland Ponies.
5	4.97^{s}			20	Stallions
23	4.8^{s}	0.2 S.E.	-	10	Shetland and Shetland-Cross Ponies
3	3.51^{p}	0.57 S.D.	1.7-4.93	111	Thoroughbreds, Standardbreds, Quarter Horses, Hacks, Polo Ponies
4	3.76^{p}		-	39	Mixed Breds and Types
	4.11^{s}			38	Thoroughbreds, Non-thoroughbreds.
14	3.88^{p}	0.035 S.E.			Racehorses (T.B.)
	3.80^{p}	0.055 S.E.			Thoroughbreds.
22			$5.2-6.1^{p}$		
24	3.68^{p}	0.143 S.D.		12	Adult Horses
	3.92^{p}	0.382 S.D.		12	Adult Horses

s = serum p = plasma

TABLE No.4

CHLORIDE CONCENTRATIONS IN EQUINE SERUM AND PLASMA CITED IN THE LITERATURE

Reference	$\bar{x}$ Cl(mEq/l)	S.D. or S.E.	Range	No. Observations	Type of Animal
23	94.9[s]	4.5 S.E.	-	9	Shetland and Shetland-Cross Ponies
25	100.8[s]		97.6-106.0	9	Thoroughbreds
3	104[p]	2.6 S.D.	94-113	111	Thoroughbreds, Standardbreds, Quarter Horses, Hacks, Polo Ponies
4	102.5[p]	0.42 S.E.		41	Mixed Breeds
6	96[p]	-	-	-	Adult Horse
16	117[p]	-	-	5	Donkeys
22	108[p]	-	-	-	-
24	99.3[p]	2.24 S.D.	-	12	Adult Horses
	100.8[p]	3.62 S.D.	-	12	Adult Horses.

s = serum p = plasma

plasma and serum are summarised in Table No.4. A wide range of values is evident; though if the mean chloride concentration of the donkeys[16] is excluded, the range is considerably reduced. Tasker[3] observed that in the horses he examined there was a trend towards higher plasma chloride concentrations in thoroughbreds than in non-thoroughbreds. He proposed that 99 to 109 mEq/l should be accepted as normal limits for equine plasma chloride levels.

Littlejohn[4], unlike Tasker[3], found no trend towards higher plasma chloride concentrations in his thoroughbreds, compared with a group of non-thoroughbreds which comprised hacks, hunters and ponies.

The inorganic phosphate concentrations measured in equine plasma and serum by other workers are presented in Table No.5. Most fall within Tasker's[3] proposed normal concentration range of 2.0 to 5.0 mgs of inorganic phosphate/ 100mls of serum. No indication of consistent differences in the inorganic phosphate content of serum and plasma was evident in the literature reviewed.

Although Stewart and Holman[1] stated that they used blood in the course of their work, it is assumed that the plasma or serum fraction of the blood was analysed, since the phosphate content of equine blood is approximately twenty times greater than plasma inorganic phosphate[28]. If blood had been analysed, Stewart and Holman[1] would have discovered substantially greater quantities of phosphate than they quoted[28].

Table No.6 summarises the concentrations of urea,

TABLE No.5

INORGANIC PHOSPHATE CONCENTRATIONS IN EQUINE SERUM AND PLASMA CITED IN THE LITERATURE

Reference	$\bar{x}$ (mgP/100ml)	S.D. or S.E.	Range	No. Observations	Type of Animal
23	1.5^{s*}	0.1* S.E.		10	Shetland and Shetland-Cross Ponies
25	4.2^{s}		3.4-4.7	9	Thoroughbreds
27	3.1^{s}	0.79 S.D.	2.0-5.6	30	Draught Horses
14	3.25^{p}	0.09 S.E.			Racehorses (T.B.)
	3.92^{p}	0.12 S.E.	-		Thoroughbreds
1	3.64^{b}		2.20-5.64	36	Clydesdales
	2.92^{b}		1.74-3.78	10	Clydesdales, Ponies, Vanners, Hunters
			2.76-3.06	2	Shetland Ponies

s = serum
p = plasma
b = blood

* This data is expressed as mEq/l.

TABLE No.6

UREA, UREA NITROGEN AND NON-PROTEIN NITROGEN IN EQUINE BLOOD, SERUM AND PLASMA CITED IN THE LITERATURE.

Reference	$\bar{x}$ mg/100ml	S.D. or S.E.	Range	No. Observed	Type of Animal
1 z	43.5[b]		30.8-59.4	36	Clydesdales.
	35.2[b]		23.5-54.5	26	Clydesdales, Ponies, Vanners, Hunters
			23.5-30.0[b]	2	Shetland Ponies
27 x	38.7[s]	6.4 S.D.	23-58	30	Draught Horses
2 x	33			1	Working Gelding
3 y			10-25[p]	112	Thoroughbreds Standardbreds Quarter Horses Hacks, Polo Ponies

b = blood
p = plasma
s = serum

x = urea
y = urea nitrogen
z = non-protein nitrogen

urea nitrogen and non-protein nitrogen determined by other authors.

Tasker[3] measured the plasma urea nitrogen concentrations in his animals and considered his results to be the normal range for equine blood urea nitrogen concentrations. If Tasker's[3] range is converted to the corresponding values of plasma urea concentrations by the use of the conversion factor (x 2.14) then his lower and upper limits of 21.4 and 51.4 mgs urea/100mls of plasma correspond closely to those obtained by Jennings and Mulligan[27].

The measurements of non-protein nitrogen concentrations made by Stewart and Holman[1] cannot be directly compared with other results quoted in Table No.6 because urea is only one of several nitrogen-containing metabolites in blood[29 30].

(iii) SPECIFIC GRAVITY

Few reports of the specific gravity of equine whole blood and serum were discovered, and no reference to the specific gravity of equine plasma was found. Published data are presented in Table No.7.

Unfortunately little indication was given of the methods used in the course of these determinations. Stewart and Holman[1], reported that they used benzene/chloroform mixtures of specific gravity range 1.000 - 1.100. To these mixtures were added drops of blood, and the specific gravity of the blood sample tested was reckoned to be that

TABLE No.7

THE SPECIFIC GRAVITY OF EQUINE BLOOD AND SERUM CITED IN THE LITERATURE

Reference	$\bar{x}$	Range	No. Observed	Type of Animal
1	$^{b}1.050$	1.047-1.060	36	Clydesdales
	$^{b}1.055$	1.045-1.070	17	Clydesdales, Vanners, Ponies, Hunters
	$^{b}1.052$	-	2	Shetland Ponies
2	$^{b}1.052$	-	1	Working Gelding
13	$^{b}1.052$	1.042-1.060	-	Cold-blooded Horses
32	$^{b}1.060$	-	-	-
33	$^{b}1.045$	1.042-1.054	14	-
2	$^{s}1.028$	-	1	Working Gelding
31	$^{s}1.0267$	1.0253-1.0281	10	-

b = blood

s = serum

of the mixture in which the drop of blood remained suspended for two minutes without sinking or rising. Eder[31] employed a gravimetric method of specific gravity determination, but no further description was available. The mean values for the specific gravity of equine blood determined by Stewart and Holman[1], Barros Santos[2] and Schalm[13] agree closely. The mean result published by Reichert and Brown[32] is much higher than the others. Since neither Scarborough[33] nor Reichert and Brown[32] specified the methods they adopted for specific gravity determinations it seems possible that some variations in results could have arisen from different methods being employed. The two mean values of equine serum specific gravity are in close agreement.

(iv) ACID/BASE PARAMETERS

Much information has been published concerning the variety of available equipment and the many methods employed to determine the acid/base status of an animal (both human and non-human), by the analysis of a blood sample or a sample of a blood constituent[34 35 36 37 38 39 40 41 42 43 44 45 46 47]. However, no choice of methods was available to measure the acid/base parameters investigated in the course of the work described in this thesis. Hence, this review of relevant literature will be confined to the values of pH, pCO_2 and bicarbonate concentration found in horse blood, or horse blood constituents.

Table No. 8 summarises the published pH values of

TABLE No.8

pH VALUES OF EQUINE BLOOD CITED IN THE LITERATURE

Reference	Mean pH	S.D. or S.E.	Range	No. Observations
3	^{v}7.38	0.03 S.D.	7.32-7.44	30
4	^{v}7.489	0.015 S.E.	-	13
24	^{a}7.386	0.0463 S.D.	-	12
	^{v}7.385	0.0410 S.D.	-	12
	^{a}7.394	0.0295 S.D.	-	12
	^{v}7.398	0.0317 S.D.	-	12
48	^{a}7.395	-	7.285-7.435	12
	^{c}7.386	-	7.300-7.430	12
50			^{v}7.20 -7.55	
51	^{a}7.47	0.006 S.E.	7.44-7.59	9
52	^{a}7.409	0.0218S.D.	7.38 -7.45	14

a = arterial blood
v = venous blood
c = capillary blood
{ = paired samples.

arterial venous and capillary blood. It is evident that a considerable range of pH values was discovered in clinically normal horses in the resting state. No distinct differences in the pH of arterial, venous and capillary blood were evident from the data presented in Table No.8.

Littlejohn and Mitchell[48] compared the pH values of

arterial and capillary blood in order to determine whether or not the two were sufficiently similar to justify using the latter as a satisfactory substitute for the former - thereby avoiding the obvious difficulties and dangers of withdrawing samples from a carotid artery. Arterial and capillary samples were collected simultaneously. After applying Docrat's criterion[49] Littlejohn and Mitchell concluded that the pH of capillary blood was sufficiently similar to that of carotid arterial blood for the former to be used as a substitute for the latter. However, it was their opinion that the practical difficulties they encountered in the course of collecting capillary blood samples rendered the technique virtually useless in veterinary medicine. The lower limit of Littlejohn's and Mitchell's[48] ranges of arterial and capillary blood pH would be considered acidotic in man[53]. No reference to a widely accepted range of normal values in the horse was discovered.

Tevik et al.[24] used the same twelve horses in their work, and presented two sets of paired arterial and venous blood pH readings. All measurements were made with the animals in a clinically normal resting state. Published values of whole blood and plasma pCO_2 are presented in Table No.9. Again Littlejohn and Mitchell[48] tested the possibility of using capillary blood as a substitute for arterial blood in the measurement of pCO_2, but the possibility was discarded for the reasons already mentioned.

From his own determinations of pCO_2 Littlejohn[52] proposed that a mean pCO_2 of 44mmHg - as opposed to the

TABLE No.9

EQUINE BLOOD AND PLASMA pCO_2 (mmHg) CITED IN THE LITERATURE

Reference	Mean pCO_2	S.D. or S.E.	Range	No. Observations
3	$v_{42.4}$	2.0 S.D.	37.8–46.0	30
4	$v_{41.1}$	1.24 S.E.	-	13
5*	$v_{47.0}$	-	-	-
24	$a_{42.9}$	7.03 S.D.	-	12
	$v_{42.7}$	5.47 S.D.	-	12
	$a_{37.9}$	3.75 S.D.	-	12
	$v_{40.0}$	4.05 S.D.	-	12
48	$a_{45.08}$	-	37.0–51.5	12
	$c_{45.46}$	-	38.5–50.0	12
50	$p_{47.0}$	-	-	-
51	a_{43}	3.6 S.E.	38 - 49	9
52	$a_{44.9}$	2 47 S.D.	42.0–50.5	14

a = arterial blood
v = venous blood
c = capillary blood
p = plasma
{ = paired samples
* = unpublished work quoted in 5.

much-quoted and much-used mean pCO_2 of 40mmHg in human blood - should be considered normal for horses, and he advocated the use of 44mmHg when calculations based on the Henderson-Hasselbach equation were being made. The values derived by others[5* 48 50 51] illustrate the higher pCO_2 of equine blood.

The bicarbonate concentrations in equine plasma and serum published by other workers are presented in Table No.10.

TABLE No.10

EQUINE PLASMA BICARBONATE CONCENTRATIONS (mEq/l) AND RELATED MEASUREMENTS CITED IN THE LITERATURE

Reference	Mean Bicarbonate concentration (mEq/l)	S.D. or S.E.	Range	No. Observations
3	$^{v}28.9$	2.7 S.D	19.5-37.0	110
4	$^{v}29.4$	0.62 S.E.	-	13
23	$^{v}30.6^{+}$	0.6 S.E.	-	10
24	$^{a}24.6$	1.66 S.D.	-	12
	$^{v}24.6$	2.40 S.D.	-	12
	$^{a}22.5$	2.17 S.D.	-	12
	$^{v}22.7$	2.45 S.D.	-	12
48	$^{a}27.20$	-	19.5-30.2	12
	$^{c}26.76$	-	18.4-29.4	12
50	$^{v}23.0^{*}$	-	-	-
51	$^{v}32.1^{+}$	1.2 S.E.	26.0-39.1	9
52	$^{a}28.5$	-	-	14
54	$^{v}28.1^{+}$	-	24 -32	-

a = arterial blood sample
v = venous blood sample
c = capillary blood sample
{ = paired samples
+ carbon dioxide content
* carbon dioxide combining power (the total carbon dioxide of oxygenised blood at pCO_2 40mmHg).

Related measurements (i.e. total CO_2, CO_2 content and CO_2 combining power) have also been included. Although the mean arterial/venous bicarbonate concentration differences determined by Tevik et al.[24] are zero in one case and only

0.2 mEq/l in another, differences within an individual animal could be masked by the manner of presentation of the results. There appeared to be a wide range of plasma bicarbonate concentrations in horses though the degree of day-to-day variation found in the individual could not be ascertained from the literature.

METHODS

THE COLLECTION AND ANALYSIS OF BLOOD SAMPLES FROM UNTREATED PONIES

Five adult Shetland and Shetland-cross ponies were used throughout this work. Two were geldings and three were stallions. One of the two geldings (Scruffy) had a unilateral parotid salivary duct fistula. The ponies ages ranged from five to sixteen years. Their body weights ranged from 164.2 kg to 214.1 kg. They were permanently housed in looseboxes, and their daily ration consisted of 4 kg of medium quality hay, and water ad libitum. In addition to hay, the fistulated pony received ½ kg of an oats/bran mixture containing 20g of sodium bicarbonate in order to replace the sodium bicarbonate lost in the saliva from the fistulated duct[55]. All had zero or low strongyle egg counts.

Blood samples were withdrawn from a jugular vein using Becton-Dickinson vacutainers and a B-D 20g needle. Care was taken to avoid stasis during collection. Samples for bicarbonate measurements were collected under mineral oil to minimise carbon dioxide loss[56 57 58]. Vacutainers containing dried heparin were used to collect blood for packed cell volume percentage, pH, pCO_2 plasma electrolytes and plasma urea determinations[59]. Before and during sampling the ponies stood quietly and care was taken to avoid causing them excitement or distress[60 61].

Determinations of pH and pCO_2 were made upon individual aliquots of heparinised whole blood, using an E.I.L.

blood gas analyser. Measurements were carried out within three to four minutes of the collection of the blood sample.

For packed cell volume percentage measurements the samples were first mixed gently and thoroughly by hand for a period of two to three minutes. Triplicate determinations were performed upon each blood sample, using Hawksley microhaematocrit tubes and a Hawkesley microhaematocrit centrifuge. The packed cell volume percentage was measured on a Hawksley microhaematocrit reader, and no correction was made for trapped plasma[20 62 63 64 65 66 67].

The remaining heparinised blood and the blood samples under oil were centrifuged for 15 minutes at 4,000 r.p.m. Centrifugation was carried out within thirty minutes of sample collection, but in the case of the samples under mineral oil it was frequently found necessary to repeat the centrifugation in order to spin down the fibrin clot which formed above the cell fraction.

The plasma was separated from the packed cells and visually examined for evidence of haemolysis. If any evidence of haemolysis was present the plasma was considered unsuitable for potassium and inorganic phosphate analyses[4] though sodium, chloride and urea determinations were still carried out. Unless the samples were required for immediate analysis the tubes were stoppered and stored at $4^{\circ}C$.

Total carbon dioxide content was measured by duplicate analyses of 1.0 ml aliquots of serum, using

the microdiffusion technique of Conway[68] and No.1 Conway units. From these results the corresponding bicarbonate concentrations were calculated using the conversion factor quoted by Wootton[29].

An EEL flame photometer was used to determine plasma sodium and potassium concentrations. The plasma was diluted 1:500 and 1:50 for sodium and potassium analyses, and, for convenience, a mixed standard solution was used to calibrate the instrument[69 70].

Plasma chloride concentrations were measured using an EEL chloride meter in accordance with the manufacturers' instructions. Plasma samples were diluted 1:50. Plasma inorganic phosphate concentrations were determined using the method of Delsal and Manhouri as described by Wootton[29]. The optical density measurements were made using a Unicam SP 500 spectrophotometer. Plasma urea concentrations were measured by Nessler's method - also described by Wootton[29]. However, in order to bring the deflection on the absorption meter of the Unicam SP 500 spectrophotometer into the range of 0.300 to 0.800 it was necessary to use 0.2ml aliquots of plasma, and the standard urea and urease solutions.

The specific gravities of blood and plasma were determined by weight, using 10 ml aliquots of well mixed blood, and plasma. The determinations were carried out at room temperature.

Analysis of variance of the results was undertaken in order to determine the significance of any differences observed between the individual ponies.

RESULTS

Summaries of the results of analyses of blood and plasma samples from the five Shetland and Shetland-cross ponies are presented in Tables 11 to 21. Complete lists of individual results are presented in Appendix No.1.

TABLE No.11

PACKED CELL VOLUMES (%)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	35.0	33.5	33.0	37.5	30.5	34.0
S.D.	1.5	3.5	2.5	4.5	3.0	4.0
n	12	15	15	14	14	70
Range	33.0 to 37.0	25.5 to 37.5	28.0 to 36.5	26.0 to 43.0	23.5 to 34.0	23.5 to 43.0

The packed cell volume percentages of the individual ponies exhibited statistically significant differences ($P<0.05$).

TABLE No.12

PLASMA SODIUM CONCENTRATIONS (mEq/l)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	136	134	133	130	135	133
S.D.	5	4	3	6	4	4
n	13	15	16	14	14	72
Range	125 to 139	128 to 138	129 to 138	120 to 138	130 to 140	120 to 140

Though analysis of variance revealed significant differences ($P<0.05$) between the plasma sodium concentrations of the individual ponies, the percentage difference between means was small, and no individual mean value differed from the overall mean by more than $\pm$ 3 mEq/l.

TABLE No.13

PLASMA POTASSIUM CONCENTRATIONS (mEq/l)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	4.05	4.75	3.85	3.75	3.80	4.05
S.D.	0.60	0.45	0.35	0.50	0.20	0.55
n	13	15	16	14	14	72
Range	3.10 to 4.95	3.80 to 5.50	3.00 to 4.40	2.70 to 4.30	3.50 to 4.20	2.70 to 5.50

Differences between the plasma potassium concentrations of the individual ponies were highly significant ($p<0.01$) but it is likely that the higher values exhibited by one pony (Jimmie) were chiefly responsible for this difference.

TABLE No.14

PLASMA CHLORIDE CONCENTRATIONS (mEq/l)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	101	100	100	99	100	100
S.D.	2	3	2	3	3	2
n	12	15	14	14	14	69
Range	99 to 106	93 to 104	97 to 104	95 to 105	95 to 105	93 to 106

There was no significant difference ($p<0.05$) between the plasma chloride concentrations of these ponies.

TABLE No.15

PLASMA INORGANIC PHOSPHATE CONCENTRATIONS (mg P/100 ml)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	3.09	3.17	3.09	3.57	2.46	3.08
S.D.	0.93	0.59	0.55	0.58	0.68	0.74
n	12	16	14	14	14	70
Range	1.28 to 4.77	1.96 to 3.98	2.08 to 4.30	2.70 to 4.38	1.30 to 4.11	1.28 to 4.77

A significant difference between ponies in their plasma inorganic phosphate concentrations was observed ($P<0.05$). This observation may be attributable to the lower values obtained from MacGowan.

TABLE No.16

PLASMA UREA CONCENTRATIONS (mg urea/100 ml)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	29.1	56.7	35.1	31.2	27.4	36.9
S.D.	7.8	11.5	15.1	10.3	5.3	15.4
n	12	15	13	14	13	67
Range	20.0 to 47.9	42.2 to 87.0	15.5 to 59.2	17.7 to 51.0	20.3 to 40.5	15.5 to 87.0

It is evident that the mean plasma urea concentration of one pony (Jimmie) was much greater than that of the others. Doubtlessly the higher values discovered in this pony were responsible for differences in plasma urea concentrations between ponies being highly significant ($P<0.01$). Appendix No.1 (vi) illustrates a consistent trend towards high plasma urea concentrations in this pony.

TABLE No.17

SPECIFIC GRAVITY OF BLOOD

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	1.050	1.050	1.056	1.056	1.047	1.052
S.D.	0.004	0.003	0.008	0.004	0.007	0.006
n	8	8	8	8	8	40
Range	1.044 to 1.056	1.046 to 1.054	1.050 to 1.069	1.047 to 1.060	1.037 to 1.058	1.037 to 1.069

Differences between the whole blood specific gravity of the five ponies were highly significant ($P<0.01$). The pony from whom the lowest specific gravity results were obtained also had persistently low packed cell volume percentages at these times (see Appendix 1 (vii)).

TABLE No.18

SPECIFIC GRAVITY OF PLASMA

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	1.026	1.026	1.027	1.024	1.027	1.026
S.D.	0.002	0.003	0.003	0.004	0.003	0.003
n	8	8	8	8	8	40
Range	1.023 to 1.029	1.021 to 1.030	1.023 to 1.031	1.017 to 1.029	1.023 to 1.032	1.017 to 1.032

The significant difference ($P<0.05$) between the plasma specific gravity values of these ponies is probably due to the lower values detected in Ben. The means, standard deviations and ranges of the results obtained from the other four ponies are very similar.

TABLE No.19

WHOLE BLOOD pH

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	7.405	7.400	7.380	7.405	7.390	7.395
S.D.	0.015	0.020	0.040	0.030	0.015	0.025
n	14	15	12	12	12	65
Range	7.380 to 7.435	7.370 to 7.430	7.280 to 7.420	7.360 to 7.470	7,370 to 7.420	7,280 to 7.470

TABLE No.20

WHOLE BLOOD pCO_2 (mmHg)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	45.5	46.0	46.0	44.5	46.5	45.5
S.D.	3.5	2.5	2.5	2.5	3.5	3.0
n	14	15	12	12	12	65
Range	38.0 to 50.5	42.5 to 51.0	42.0 to 50.0	40.0 to 49.0	38.0 to 51.0	38.0 to 51.0

TABLE No.21

SERUM BICARBONATE CONCENTRATIONS (mEq/l)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	28.7	27.9	27.8	26.9	28.2	27.9
S.D.	2.0	1.2	1.0	2.0	1.2	1.6
n	14	20	17	15	12	78
Range	25.9 to 32.8	25.6 to 29.5	25.7 to 29.5	23.7 to 29.9	26.4 to 30.5	23.7 to 32.8

There were no statistically significant differences ($P<0.05$) between the five ponies with respect to venous pH, pCO_2 and serum bicarbonate concentrations.

DISCUSSION

(i) VENOUS PACKED CELL VOLUME PERCENTAGE

The results obtained from the ponies accord with those reported by authors who investigated other types of cold-blooded equines[1 2 4 7 9 13 14] which were mainly,but not exclusively, represented by draught animals. The mean packed cell volume percentage of all the ponies was higher than that of the ponies studied by Deavers et al.[17]. It was also higher than the packed cell volume percentages of Stewart's and Holman's[1] two Shetland ponies, though a paucity of results from the latter made a very meaningful comparison impossible.

Although Tasker[3] failed to observe any significant difference between the venous haematocrit of his thoroughbreds and non-thoroughbred horses, it is probable that because all were "riding type" horses they would be doing similar work. It is possible, too, that some of the non-thoroughbreds could have had some thoroughbred ancestry.

Littlejohn[4], Sréter[14] and Marcilese et al.[9] all reported statistically significant differences between hot and cold blooded horses. Schalm[13] emphasised that when assessing a haematocrit value, it should be known whether the horse in question was hot or cold blooded.

This raises the basic question of why such a difference occurs, and what factors can influence the packed cell volume percentage.

McLeod and Ponder[71] believed the higher packed cell volume percentages of thoroughbreds to be basically a

genetic phenomenon. Marcilese et al.[9] stated that the differences in both venous and "whole body" haematocrits between thoroughbreds, saddle horses and Percheron-cross draught horses were related to the physiological characterstics of the breeds, but they did not venture an opinion of whether or not these differences were inherited or acquired.

A possible correlation between the age of a horse and its haematocrit was investigated by several workers[8 9 11 72]. Archer[8] found no correlation between the age of a horse and its haematocrit. His narrow range of results from a large number of horses whose ages ranged from birth to adulthood illustrate his conclusion. Marcilese et al.[9] stated that only in the first few months of life, and in advanced years was age likely to influence the venous haematocrit. Medeiros, Martins, Ferri and Barcelos[72] - working exclusively with thoroughbreds - also studied the influence of age on the haematocrit and found that in early life the haematocrit was lower than that of the mature animal. The increase towards the packed cell volume percentage of the adult thoroughbred was found by Medeiros et al.[72] to commence when the foals were approximately three weeks old. They appeared to relate this increase to age alone, and no other causes were suggested.

Collins[11], noted that the packed cell volumes of his thoroughbreds were higher in horses of three years and over than in animals below this age, but from his knowledge of their management he attributed the higher packed cell

volume percentages of the older age group primarily to their greater degree of physical fitness, as opposed to age alone.

Thus, opinions differed somewhat upon the extent to which the age of a horse influenced its venous haematocrit, but evidence strongly suggests that in the mature years of a horse's life age differences alone apparently had no affect upon the haematocrit. Hence, any differences in the results from the five Shetland ponies, whose ages ranged from five to sixteen years old, were unlikely to be due to age, since the age range did not encroach upon immaturity nor advanced years. Furthermore, there was no correlation between ages and the mean venous packed cell volume percentages of these ponies.

Littlejohn[4] quoted Neser as stating that the higher packed cell volume percentages observed in thoroughbreds result primarily from regular exercise, rather than from their breed, age or sex. This partly supports Collin's[11] theory that the fitter the horse, all other things equal, the higher its haematocrit will be, though presumably "fitness" in Neser's context implied speed as well as stamina, for the work of a draught horse, albeit slow, requires strength and sustained effort, if not speed. McLeod, et al.[25] believed that the plane of nutrition influenced the haematocrit.

That the venous haematocrit of the five Shetland ponies resembled those of draught horses more closely than those of thoroughbreds was expected, since the conformation

and management of the ponies resembled those of draught animals, and differed greatly from those of the thoroughbred horse.

It has been often reported, and is widely accepted, that the manner of handling equines prior to and during blood sampling can profoundly affect the packed cell volume percentage of the sample[60 61]. The horse is normally a nervous animal, well adjusted to the "fright and flight" phenomenon, and a sudden rise in the level of circulating adrenalin is rapidly followed by an increased haematocrit[61]. Whilst splenic contraction is generally believed to be mainly responsible for this increase in haematocrit[18 61], evidence suggests that other physiological mechanisms causing a redistribution move of red cells to the peripheral circulation are evoked[12 61]. The use of a twitch and/or tranquilising drugs for restraint, undue excitement and/or fear, and exercise have all been shown to effect changes in the venous haematocrit[12 14 15 18 60 61 73] and this is why, to avoid distress, the Shetland ponies were restrained by haltering only. Furthermore the ponies had always rested before blood samples were taken.

The use of jugular catheters for frequent blood sampling was found by some workers to be advantageous[7 60 73 74]. This was not considered worthwhile in the case of the Shetland ponies, where, in the course of this work many days often elapsed between sampling from individual animals. Also, the use of Becton-Dickinson vacutainers, as opposed to syringes, made possible the use of fine gauged needles which caused minimum tissue trauma, and discomfort. Further-

more the ponies were accustomed to the procedure of sampling and seldom appeared disturbed.

Many factors which are capable of affecting the results of packed cell volume determinations exert their effect after the withdrawal of the sample from the animal. Some of these factors can at least be minimised, if not completely eliminated, by good technique.

The erythrocyte sedimentation rate of equine blood is high compared with that of other species[12 65] so that adequate mixing of the sample before filling the macro- or micro-haematocrit tubes is especially important, particularly after a long time lapse between the collection of the sample and the packed cell volume determination. Triplicate determinations of the packed cell volume percentage of all blood samples collected from the ponies thus served as a check of adequate mixing.

It has been observed that the tonicity of the anticoagulant could cause swelling or crenation of the erythrocytes[20 63]. However, the anticoagulant in the B-D vacutainers, 143 USP units of freeze-dried heparin, exerted no osmotic effect. The temperature of the blood at the time of centrifugation has been shown to affect the extent of packing of erythrocytes[20 65]. Lauder[20] demonstrated that over the range of 5°C to 25°C the higher the temperature the greater the degree of packing and hence the more accurate was the packed cell volume reading.

Varying the proportion of the microhaematocrit tube filled with blood has been implicated as a cause of error[62 75 76 77 78]. Love and Kirkham[62] found that discrepancies

of about 1.5% arose from tubes 1/3, 2/3 and completely full of blood. These differences were statistically highly significant ($P<0.01$). The percentage of trapped plasma was least in the red cell columns of tubes containing least blood, because the mean effective radius of centrifugation, taken as the distance from the centre of the centrifuge to the centre of the erythrocyte column (M.E.R.C.), and hence the relative centrifugal force (R.C.F.), were greatest. [R.C.F. (dynes) = $1.119 \times 10^{-5} \times (\text{r.p.m})^2 \times \text{M.E.R.C.}$] Hlad and Holmes[67] concurred with Love and Kirkham[62] and discovered that the time of centrifugation exerted an effect upon the final result too. Other workers believed that errors arising from varying the length of the blood column in the microhaematocrit tubes were very small, and to the point of being undetectable[63 64].

In the course of the work carried out with the Shetland ponies the microhaematocrit tubes were approximately ¾ filled with blood. The results could only be read to be nearest 0.5% on the microhaematocrit reader, so that variations of less than this were undetectable. Furthermore errors could arise due to a parallax effect between the top of the red cell column and the line on the microhaematocrit reader, so that the shorter the red cell column the greater was the % error in reading. Whilst the greatest possible accuracy was aimed for, reading errors of 0.5% could arise however much care was taken. In the context of the work undertaken with these ponies, and in view of other sources of error described in this discussion - many

not completely avoidable - the significance of any error arising due to the degree of filling of the microhaematocrit tubes was disregarded.

Several authors examined the problem of plasma trapping in the erythrocyte column[20 62 66 67], and others applied correction factors obtained from published work[7 9 73]. Hiroto[66], one of the earliest workers to concern himself with this problem, discovered a mean value of 1.5% for plasma trapped amongst the erythrocytes of equine blood after centrifugation.

Lauder[20] found that after samples of equine blood had been centrifuged at 1,500g for 60 minutes at 25°C there was 2.8 ± 0.3% (mean ± SD, n = 32) plasma "trapped" amongst the packed red cells. Changing the ratio of cells:plasma in samples was found not to change the percentage of plasma "trapped" though Lauder[20] observed that the narrower the bore of the microhaematocrit tube, the higher was the packed cell volume percentage. Contrary to Lauder's finding was that of Hlad and Holmes[67] who stated that the quantity of plasma trapped in the erythrocyte column was affected by the haematocrit.

Lauder[20] discovered that in any microhaematocrit blood sample the amount of plasma trapped increased from the base of the tube to the cell/plasma interface. This observation accords with that of Love and Kirkham[62].

The time and force of centrifugation of blood samples from the ponies were constant. Because the error arising from plasma trapping in the microhaematocrit tubes

was likely to be very small[18 65], and because the packed cell volume percentages of the Shetland ponies were intended primarily for comparative purposes[67 79], no correction factor was applied.

(ii) ELECTROLYTES AND UREA

The total mean plasma sodium concentrations in these ponies was lower than all those listed in Table No.2 with the exception of that quoted by Alexander[23] and the lower limit of the range of plasma sodium concentrations exhibited by the ponies was much lower than that discovered by workers who published the ranges of their results[1 3 16 22]. Before attempting to ascertain the reason(s) for these low results it was obviously necessary to eliminate errors in analytical technique. In an effort to ensure that the flame photometer was not malfunctioning frequent checks of the stability of the instrument were made throughout series of analyses.

Checks of the standard solution were made using another standard solution containing 150 mEq/l of sodium, prepared by a colleague. Agreement was found to be within ± 1 mEq/l. When sufficient quantities of plasma were available duplicate determinations of the sodium concentrations were performed. Agreement was again found to be within ± 1 mEq/l.

The total mean plasma sodium concentration differed from Alexander's[23] mean by only 1 mEq/l. Since both results were derived from Shetland and Shetland-cross ponies the question arises of whether the comparatively low sodium

levels are a breed characteristic, and it was unfortunate that no further reports of plasma sodium concentrations in such ponies were discovered. However, the possibility that the age, diet and management could exert an effect upon the concentration of this electrolyte was worthy of consideration.

The ages of the five Shetland ponies ranged from five to sixteen years. However, Amrousi and Soliman[80] showed that a horse's age did not appear to affect its plasma electrolyte concentrations. Moreover the mean values from the youngest and the oldest ponies (MacGowan and Jimmie respectively) did not fall at the extremes of the range of means.

It seemed unlikely that the diet of these five ponies exerted a significant effect upon their plasma sodium concentrations. Many of the mean values quoted in Table No.2 were derived from horses fed solely on hay. The notable exceptions were the working thoroughbreds, and their mean plasma sodium concentrations did not markedly differ from those of other groups of horses.

In another publication Alexander[81] reported upon differences he observed in the range of plasma sodium and potassium concentrations between ponies who were permanently stabled, and who consequently received virtually no exercise, and a group of vigorously exercised hunters. The mean plasma sodium concentrations of the stabled ponies was approximately 20 mEq/l greater than that of the hunters, and the range of sodium concentrations in the stabled ponies

was approximately seven times greater than that of the hunters. However, this observation needs cautious interpretation. Because Alexander[81] was not able to vigorously exercise the ponies nor stable and rest the hunters he was able to prove that exercise, or lack of it, was responsible for the differences he observed. Furthermore, whilst it is not suggested that a discrepancy in the sample numbers wholely accounts for the differences observed by Alexander[81] it is worthy of note that the number of observations made of the stabled ponies was approximately three times that made of the hunters, so that a greater range of values in the larger population would be expected.

The total number of measurements of plasma sodium concentrations in the five Shetland and Shetland-cross ponies used for the work described in this thesis was seventy-two. This is similar to Alexander's[81] sample number of sixty-seven, but the concentration range observed by Alexander[81] was greater than twice the range of values from the five Shetland and Shetland-cross ponies, and the mean plasma sodium concentration of Alexander's ponies was nearly 10 mEq/l greater than that of the five Shetland-type ponies. Unfortunately Alexander[81] failed to specify the number of ponies he examined. It was deduced that permanent stabling and lack of exercise probably did not solely account for the comparatively low plasma sodium concentrations observed in the ponies used in the work described in this thesis.

None of the work so far described and discussed has excluded the possibility of the comparatively low plasma

sodium concentrations of the five Shetland and Shetland-cross ponies being a breed characteristic. Of the five ponies, one was known to be pure Shetland, two were judged likely to be pure Shetland, and the remaining two were considered to be of mixed, but predominantly Shetland breeding. If a tendency to a lower-than-average plasma sodium is inherited, and if this characteristic is dominant, then there is an excellent chance that it would be manifest in most purebred Shetlands, and in many Shetland crosses. Unfortunately no other Shetland ponies were available for examination. A change in the system of management of these ponies would have been the obvious way of investigating whether permanent stabling and lack of exercise influenced the plasma sodium concentrations of these ponies.

It was concluded that the results obtained from the five ponies were a true reflection of their normal plasma sodium concentration ranges. It was unsatisfactory to be unable to show why these values differed from those obtained by other workers, but no definite conclusions could be drawn, either from the work of others or from the work reported in this thesis. However, since the system of management of these ponies was constant throughout the whole time they were used, the results were valuable in determining the extent to which experimental treatments disturbed the normal electrolyte concentrations.

The commonly accepted normal range of plasma potassium concentrations in human subjects is 3.50 to 5.50 mEq/l[53]. No universally accepted normal range for equine plasma

potassium concentrations appears to have been adopted, though Tasker[3] proposed that equine plasma potassium concentrations should normally fall within the limits of 2.40 to 4.70 mEq/l. The range obtained from the five Shetland and Shetland-cross ponies of 2.70 to 5.50 mEq/l had higher limits than those suggested by Tasker[3] whose proposed normal values were derived partly from his study of available relevant literature, and partly from the results he himself determined experimentally. Tasker's[3] lowest result of 1.7 mEq/l appears grossly abnormal, and lies well beneath his own defined lower limit of normality though he reported that all the horses he studied were apparently healthy. Unlike Alexander[81] and Soliman and Nadim[82], Tasker[3] found no evidence of management and exercise influencing plasma potassium concentrations in his horses.

Excluding Bernstein's[22] high results, a general trend towards the potassium concentration in serum being higher than in plasma was evident from Table No.3. Littlejohn[4] was able to demonstrate this difference using paired serum and plasma samples obtained simultaneously from individual horses. Littlejohn[4] stressed that this difference could be masked by errors in technique, including delay in the separation of the plasma or serum from the red cell mass, and the use of an anticoagulant (in the case of plasma samples) which contained potassium. Haemolysis could be a considerable source of error too. These errors all cause increases in plasma potassium concentrations.

Pertinent to the discovery that in the blood of an individual horse at any given time the serum potassium concentration was higher than that in plasma is the work of Pannal and Rossi[59], who reported that the same was usually true in paired plasma and serum samples from man. They also emphasised the errors which could arise as a result of haemolysis. Pannal and Rossi[59] advocated the use of plasma rather than serum for potassium estimation because in their opinion the use of serum could conceal hypokalaemia when the plasma potassium level was below the lower limit of the accepted normal range.

Pannal and Rossi[59] quoted Phleiderer's postulation, and his supporting evidence, that thrombocyte destruction during coagulation releases potassium. Whilst proof that an identical process occurs in equine blood is lacking, Littlejohn's[4] observation provided strong evidence in favour of potassium release at some stage between the collection of a blood sample and the analysis of the serum therefrom.

All the mean values of the ponies' plasma chloride concentrations fell within the normal range of equine plasma chloride concentrations proposed by Tasker[3] though four of the ponies yielded individual results which were below Tasker's proposed lower limit of normality. No result ever exceeded Tasker's upper limit of 109 mEq/l.

Since the ranges of plasma chloride concentrations from all of the ponies were similar it was considered that the normal range for these animals was lower than

Tasker's[3] and no special significance was attached to this difference, especially as the values from these ponies were similar to those found by some other workers[4 24 25]. Though McLeod et al.[25] published concentrations of serum chloride, no reference was discovered which suggested that serum and plasma chloride concentrations differed.

No publication reviewed indicated that differences might arise between the inorganic phosphate content of serum and plasma. Neither was there any suggestion that plasma and/or serum inorganic phosphate concentrations in thoroughbred horses and non-thoroughbred horses and ponies consistently differed.

Most of the results obtained from the Shetland and Shetland-cross ponies fell within Tasker's[3] suggested normal concentration range of 2.0 to 5.0 mg P/100 ml, and were similar to those obtained by Stewart and Holman[1] from their two Shetland ponies.

The mean plasma urea concentrations of four of the five Shetland ponies were similar. The reason for Jimmie's high plasma urea levels is unknown. This discovery was not investigated further.

Since Tasker's[3] range of 10 - 25 mg urea nitrogen/100 ml plasma is equivalent to 21.4 to 51.4 mg urea/100 ml plasma, the range of values determined in the other four ponies is similar both to Tasker's proposed normal range and to the results of Jennings and Mulligan[27]. Campbell and Watts[83] discovered that in cattle which showed persistently high blood urea levels concurrent fluid and erythrocyte disturbances were frequently present. These included hypo-

chloraemia, which was often very severe, especially when blood urea levels were very high, low plasma sodium concentrations, and, sometimes, low plasma potassium concentrations. No evidence of hypochloraemia or hypokalaemia was found in this pony, and plasma sodium was not markedly lower than in the other four ponies.

Plasma was used to measure the urea concentrations in the five Shetland and Shetland-cross ponies, chiefly in order to avoid the turbidity which can develop when Nessler's reagent is added to a filtrate containing glutathione and ergothioneine[29].

(iii) SPECIFIC GRAVITY

Since erythrocytes have a higher specific gravity than plasma it would be expected that low packed cell volume percentages would be conducive to a low blood specific gravity value, and this was observed in MacGowan. Other than a general trend towards higher packed cell volume percentages corresponding with higher specific gravity values, there was no obvious direct relationship between packed cell volume percentages and the whole blood specific gravity values of these ponies.

The total mean blood specific gravity value obtained from the five ponies was identical to that quoted by Schalm[13] and Barros Santos[2]. It was also identical with the mean value discovered by Stewart and Holman[1], in two Shetland ponies, and it was similar to the results they obtained from other cold blood horses and ponies. However the range of results obtained from the five Shetland and

Shetland-cross ponies was wider than those quoted by Stewart and Holman[1] and Schalm[13].

Unfortunately no reference to the specific gravity of equine plasma was discovered, so it was impossible to compare the results with any others. However, Barros Santos[2] and Eder[31] both published very similar results of their measurements of serum specific gravity, and the mean plasma specific gravity values of these ponies closely resemble the results obtained by these two workers. It was deduced that the variation in plasma specific gravity observed in the individual ponies was the most likely reason for the lack of a direct relationship between the packed cell volume percentages and the specific gravity of the samples.

(iv) ACID/BASE PARAMETERS

From the results of venous blood pH measurements in these ponies, and from the results published by others, with one exception[4], it appeared that the mean pH of equine venous blood was lower than the often-quoted normal human mean value of 7.42.

In view of the difficulties and sources of error reported by Littlejohn and Mitchell[48], no attempt was made to obtain capillary blood samples from the Shetland ponies. Because the hazards of obtaining arterial blood samples were considered to outweigh the advantages of a knowledge of arterial blood pH, no arterial blood samples were collected either. The collection of venous blood from

the jugular vein was easy for the operator, and relatively painless and risk-free for the ponies. Venous blood was found to be satisfactory for comparing differences in acid/base parameters between the individual ponies, and between these ponies and animals investigated by other workers. At a later stage the results obtained were used to study the effect of experimental treatments, when each pony was used as his own control.

The mean pCO_2 values of the five Shetland ponies were in close agreement. The ranges of the values were also similar. The mean pCO_2 pressures in the venous blood of these ponies were somewhat higher than most of those listed in Table No.9. The results from all five ponies provided further evidence for the normal pCO_2 of equine venous blood being greater than that of man. However, the mean values from these ponies are higher than Littlejohn's[52] proposed normal mean.

The length of time which can elapse after withdrawal of the blood samples before changes in pH and pCO_2 reach measurable dimensions does not appear to have been reported. The E.I.L. blood gas analyser used in the course of the work with the Shetland ponies measured pH to 0.005 unit, and pCO_2 pressures to 0.5mmHg. Samples were injected into the instrument within 3 to 4 minutes of their collection, so that if delay in performing measurements was a source of error it was minimised by rapid transference of the blood sample to the blood gas analyser. Exposure of the samples to atmospheric air was also minimal. The transfer of blood

from a hitherto sealed tube into a syringe before it could be injected into the pH and pCO_2 cuvettes was accomplished very rapidly by inserting a needle via the seal deeply into the blood in the vacutainer. Care was necessary to avoid bubbling air through the blood samples. These precautions in sampling and analytical techniques were believed to minimise avoidable error.

All ponies had similar ranges and mean values of serum bicarbonate concentrations. The results from these ponies compared closely with most of the bicarbonate concentrations listed in Table No.10[3 4 48 52], though some of the data listed in this table were derived from arterial and capillary blood, as well as venous blood. The results obtained by Tevik et al.[24], are low compared both with those from the five Shetland ponies and the others listed in Table No.10.

Where methods of quantitative analysis of bicarbonate vary, differences in results are likely, and indirect determinations, which usually consist of calculating bicarbonate levels from direct measurements of pH and pCO_2 and applying the Henderson-Hasselbach Equation, or using a nomogram (also based on the Henderson-Hasselbach Equation), will yield different values to those determined by direct measurement[52]. Thus, for accurate comparisons, analytical techniques must be identical. The use of human nomograms for assessing equine acid/base parameters is also a source of considerable error[52].

All blood samples for bicarbonate determinations,

collected during the course of this work were collected under mineral oil in a sealed tube. Paulsen[56] published some controversial viewpoints on the value of paraffin oil for protecting blood samples intended for bicarbonate analysis. He quoted Kubie[84] who declared that paraffin oil was not ideal for this purpose because carbon dioxide was more soluble in paraffin oil than in water, and there was also the added difficulty of pipetting off the plasma or serum without some paraffin oil entering the pipette. Paulsen[56] also quoted Peters and Van Slyke[57], who, contrary to Kubie[84], stated that blood kept under paraffin oil incurred no great carbon dioxide loss, but centrifugation caused a loss whether or not the sample was protected by paraffin oil. Harrison[58] was reported to have found that stoppering the tubes containing the samples afforded adequate protection against carbon dioxide loss, and this obviated the use of oil.

Paulsen[56] himself discovered that stoppering the container immediately after the collection of the sample was as effective a protection against carbon dioxide loss as paraffin oil, provided the container was completely filled. He observed that refrigeration of the samples reduced carbon dioxide loss, and confirmed that centrifugation was a major cause of loss. Paulsen[56] showed that unless the plasma or serum was promptly separated from the red cells, in vitro erythrocyte respiration increased the carbon dioxide content of the serum and/or plasma. Furthermore, he advocated heparin as the anti-

coagulant of choice if plasma was being used, since it is neutral, carbonate-free, and, because it exerted no osmotic effect, it did not cause the redistribution of ions between the erythrocytes and the plasma. Paulsen[56] found no differences in the bicarbonate concentrations of paired serum and plasma samples provided both were treated and analysed similarly.

As far as was possible Paulsen's[56] recommendations were adhered to in the work with the Shetland ponies. Whole blood used for pH and pCO_2 measurements was heparised, though serum, as opposed to plasma, was used for bicarbonate determinations. Since the vacutainers did not fill completely the covering of mineral oil was advantageous, and because the stopper had to be removed before centrifugation, the blood was covered at all times between collection and analysis. Centrifugation could not be avoided, and repeated centrifugation was sometimes necessary.

It would seem logical that since paraffin oil had been used to protect samples for bicarbonate determinations from carbon dioxide loss, the blood for pCO_2 measurements should have been likewise protected. There were two principal reasons for this not being done. Firstly, there was the risk that oil could have been introduced into the pCO_2 cuvette of the blood gas analyser. It proved easy, after a little initial practice, to avoid introducing oil into the Conway unit from a pipette, and even if a small globule was introduced, nothing worse than an equally small error in the bicarbonate concentration determination would

have arisen. Had any oil entered the pCO_2 cuvette erroneous results would have been obtained, or a complete loss of function of the pCO_2 cuvette system could have occurred.

Secondly, the vacutainers containing mineral oil contained no anticoagulant and thus there was a risk of coagulation causing a blockage of the very fine tubes of the pCO_2 cuvette. This would necessitate the dissembling and unblocking of the cuvette. The time lapse of 3 to 4 minutes between the collection of the sample and its introduction into the cuvette, plus the time the blood remained in the cuvette, was adequate for coagulation to take place.

The advantages of using vacutainers rather than syringes for blood sampling have already been described and discussed.

PART 2

URINE

INTRODUCTION

So far as could be ascertained, studies upon equine urine are fewer in number than those upon equine blood. The earliest account of the volume of urine passed by horses was that by Zuntz and Hagemann, quoted by Dittmer[5]. These two workers investigated thirty-four horses of mean weight 420 kg, and discovered that the mean urine volume under normal conditions was 12 ml/kg/day. Swenson[85] published Ellenberger and Scheunert's observation that the volume of urine voided by their horses ranged from 3 to 18 ml/kg body weight/day.

Tasker's[86] investigation of the intake and output of water, sodium and potassium in normal horses of approximately 1,000 lbs weight revealed that an average of 5.5 litres of urine containing 4.9% water was passed daily.

Tasker[87 88] reported that under conditions of dehydration approximately 2 litres of urine - the so-called daily obligatory urine loss - were passed by his horses.

Nicholson[89] worked with a group of five Shetland and Shetland-cross ponies, and measured the volume, pH, and the sodium, potassium and chloride concentrations in the urine the ponies voided over a twenty-four hour period. From the urine volume, and the concentration of these electrolytes, the urinary losses/24 hours of sodium, potassium and chloride were calculated. Nicholson[89] did not measure the water intake of these ponies but the total

mean urine volume passed/24 hours was 4,240 ml ± 1940 S.D., (n = 139). Individual mean volumes ranged from 3,600 mls to 5270 mls.

Two references to Ellenberger and Scheunert's measurements of equine urine specific gravity were found[5 26]. They discovered a normal range of 1.025 to 1.060, and a mean of 1.040. Nicholson[89] discovered that though there was a tendency towards an inverse relationship between volume and specific gravity in the samples he examined, no simple relationship was apparent.

Working with human urine, Galambos, Garland - Herndon and Reynolds[90] made several observations relevant to all urine specific gravity determinations. Their first point referred to the human error in the reading of the hydrometer. Secondly, they stressed the often-overlooked fact that hydrometers are calibrated at one specific temperature, and since temperature variations affect the density of liquids corrections need to be applied for this. Thirdly, these workers emphasised that the specific gravity of a fluid indicates its density, but not necessarily the amount of solids dissolved. Though Roberts' coefficient (K) can be used to estimate total solids, by substitution in the equation TOTAL SOLIDS = 1,000 K (S.G. - 1.00)/litre, this, in biological fluids of variable composition, should be regarded as only the roughest of guides. In complex solutions such as urine, where each of the solutes exerts its own effect upon specific gravity, and where the relative concentrations of these solutes are unknown, specific gravity cannot give precise quantitative information on the

concentrations of dissolved substances. Galambos et al.[90] recommended osmolarity determinations as a far better indication of the degree of concentration of urine.

Several references to the pH of equine urine have been published. Tasker[26] quoted Ellenberger and Scheunert's discovery of a normal pH range of 7.0 to 8.0. Debackere and Laruelle[91] reported finding the urine pH in specimens from horses used for experimental purposes strongly alkaline, and that from racehorses acid or neutral, but no further details were furnished, and no explanations were offered.

The mean pH of urine specimens collected from five ponies by Nicholson[89] was 8.0 ± 0.5 S.D. (n = 139). Individual means ranged from 7.8 to 8.2, and though the total mean pH value was just within the upper limit of Ellenberger and Scheunert's range, obviously many of Nicholson's[89] measurements must have fallen above the upper limit quoted by Ellenberger and Scheunert. Nicholson[89] considered that a knowledge of the pH of urine was merely a useful guide to hydrogen ion concentration, rather than an accurate reflection, since other ions, beside hydrogen ions, exert an effect upon pH.

Tasker[26 86 87 88 92] and Nicholson[89] studied electrolyte excretion in horses. Tasker[86] studied clinically normal adult horses of approximately 1,000 lbs weight, which were fed 10 kg of hay daily, and allowed drinking water ad libitum. The sodium content of the hay was 32.9 mEq/kg giving a total daily sodium intake of 329 mEq (drinking water was disregarded as a source of sodium and

potassium). The potassium content of the hay was 393 mEq/kg - giving a total daily sodium intake of 3930 mEq. Mean urinary sodium excretion/24 hours was 7.1 mEq, or 2.1% of the intake, and mean urinary potassium excretion/24 hours was 2169 mEq, which represented 55% of the intake.

Nicholson's[89] investigation of the percentages of the daily sodium and potassium intake excreted in urine yielded markedly different results to those obtained by Tasker[86]. Nicholson[89] estimated that his ponies each ingested approximately 260 mM/day of sodium, and 1330 mM/day of potassium. A mean urinary sodium excretion of 140 mM/day represented 54% of the intake, and a mean urinary potassium excretion of 1320 mM/day represented 99%. Thus, though Tasker[86] could only account for 2.1% of the daily sodium intake and 55% of the daily potassium intake in terms of urinary loss. Nicholson[89] found that over half the daily sodium intake and, virtually all the daily potassium intake was excreted via the kidneys.

Nicholson[89] did not estimate chloride intake, but he did measure urinary excretion of this anion, and the total mean loss in urine passed over a 24 hour period by his ponies was 737 mM $\pm$ 350 S.D. (n = 139). Mean daily urinary losses from the individual ponies ranged from 640 mM to 814 mM. He discovered that the concentration ratio of sodium: potassium: chloride was approximately 1:10:5 in the urine from his ponies.

When Tasker[88] withheld food and water from his horses

for 8 days he observed an increased urinary sodium excretion. He attributed this to the sodium concentration in the animals' digestive fluids being greater than that in the animals' normal diet. He considered that when food and water were withheld, fluid was reabsorbed from the gut to replace water and electrolytes normally acquired in the diet. To avoid a rise above normal of plasma sodium, most of the sodium absorbed from the intestinal tract was excreted in urine. Conversely, urinary potassium was observed to decrease when dietary potassium intake ceased. Because the decrease in urinary potassium excretion that Tasker[88] observed was gradual, he believed that equine renal potassium regulation was not very closely regulated. Tasker[88] quoted Sisson and Grossman[93] who intimated that the capacity of the digestive tract of an adult horse ranges from approximately 120 to 150 litres, and that the water content of the gut is greater than 80%. Hence Tasker[88] postulated that one of the important functions of the equine digestive tract was the maintenance of a reservoir of water at least some of which could be utilised to offset potential dehydration.

In order to establish the normal ranges of the volume, pH, specific gravity and the quantities of selected constituents of the urine voided over 24 hour periods by the five ponies used for the work described in this thesis, urine samples were collected and analysed. The results obtained were also compared with those published by other workers.

METHODS

THE COLLECTION AND ANALYSIS OF 24 HOUR URINE SAMPLES FROM UNTREATED PONIES

The five ponies used for urine collections were those from whom blood samples were obtained. Diet and general management have already been described, though the collection of urine necessitated the ponies standing in stalls for the duration of the collection.

A funnel and harness designed by Warwick[94] was used to direct the urine into a plastic collecting bottle of approximately nine litres capacity, which was placed in a cool covered well at the side of the stall. The collecting bottles could not capsize. The ponies were well accustomed to standing in harness.

Immediately after the collection period the urine was well mixed before measurements and analyses were undertaken. The volume was measured to the nearest 10 ml using a 2 litre measuring cylinder. Specific gravity was determined using a hydrometer calibrated to 0.001 unit, and pH was measured to 0.05 unit with either a Marconi or a AML Model 40 pH meter. An EEL flame photometer was used to determine sodium and potassium concentrations. A dilution of 1:500 was usually adopted for sodium measurements, unless sodium concentrations were below 12 mEq/l, when a dilution of 1:250 was found more suitable. For potassium determinations a dilution of 1:5000 was made. An EEL chloride meter was used to measure urinary chloride concentrations, and 10 ml aliquots of a 1:50 dilution of urine

were used for analyses.

The inorganic phosphate concentrations of the samples were measured by the method of Delsal and Manhouri as already described[29]. It was customary to use 0.2 ml aliquots of undiluted urine, but when the inorganic phosphate concentrations were greater than approximately 14 mgP/l it was necessary to dilute an aliquot of the original urine sample and then repeat the initial procedures before continuing with the colorimetric determinations. The absorption was read in a Unicam SP 500 or SP 600 spectrophotometer at 880 nM. A tungsten lamp was the light source in the SP 500, and a red filter was used in the SP 600.

Ammonium, urea and "total nitrogen" concentrations were determined by Nessler's method as described by Wootton[29]. To avoid turbidity developing simultaneously with colour when Nessler's reagent was added, it was found necessary to add two drops of a 2% iodine/3% potassium iodide mixture to the blank, standard and each test sample[29]. This did not affect the intensity of the colour which developed when Nessler's reagent was added, and obviated the need for cooling the mixtures.

Net acid/base excretion in urine was measured using a modification of Jørgensen's method[95]. 1.000 N hydrochloric acid was added in multiples of 4 mls to 40 ml urine. After boiling, the acidified urine was allowed to cool to room temperature. The volume was restored to the original volume of urine (i.e. 40 ml) plus the volume of

hydrochloric acid used to reduce the pH to 4.00 or below, with distilled water. Duplicate blanks and duplicate test samples were titrated, as a check of accuracy. Titres in each case agreed to 0.05 mls. The two blank samples each comprised 50 mls of 7% to 8% formaldehyde solution plus one quarter of the total volume of acid added to the original 40 ml aliquot of urine. The test samples each consisted of 50 ml of 7% to 8% formaldehyde solution plus one quarter the total restored volume of the boiled acidified urine.

In a further series of urine collections and analyses, the urine passed over a twenty-four hour period was collected in the form of the individual samples voided. The collection apparatus was modified by the incorporation of an electronic temperature sensing device designed and constructed by Nicholson and Warwick[96]. This device was incorporated for three reasons. Firstly, the exact times of urine voidance could be easily calculated from the marks on the tracing along the smoked kymograph paper. Secondly, since the ponies were left unattended for short times during the day, and for circa 10 hours overnight, it was possible to ensure that the urine in the collecting bottle represented a single voiding only. Thirdly, it conferred the less essential but nevertheless practical advantage that it was merely necessary to examine the tracing mounted on a drum above the stall instead of examining the collector itself, to ascertain whether urine had been passed.

The individual samples were measured and analysed

using the methods already described. Hence, both the concentrations and total quantities of electrolytes and urea were determined.

RESULTS

The summarised results of analyses of urine samples collected from the five Shetland and Shetland-cross ponies are presented in Tables 22 to 33. All results were subjected to analysis of variance in order to determine the significance of any differences observed between ponies.

Complete lists of individual results are presented in Appendix No.2.

The results of the analyses of single samples voided by ponies over 24 hour collecting periods are presented in Tables 34 to 37.

TABLE No.22

URINE VOLUME PASSED IN 24 HOURS

(ml)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	5450	3180	3880	3340	4480	4080
S.D.	1760	1160	1550	1430	1390	1700
n	25	22	23	25	17	112
Range	1920 to 8760	1550 to 6640	1690 to 6530	1540 to 6390	2000 to 7820	1540 to 8760

TABLE No.23

URINE VOLUME PRODUCED ml/kg BODY WEIGHT/24 HOURS

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	30.5	19.4	21.4	18.4	20.9	22.2
S.D.	9.9	7.1	8.5	7.9	6.5	9.2
n	25	22	23	25	17	112
Range	10.2 to 49.0	9.4 to 40.4	9.3 to 36.0	8.5 to 35.2	9.3 to 36.5	8.5 to 49.0

Differences between ponies in the volumes of urine voided/24 hours were highly significant ($p<0.01$). Urine volume/kg body weight/24 hours was very similar in four of the ponies.

TABLE No.24

SPECIFIC GRAVITY OF URINE SAMPLES

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	1.019	1.030	1.027	1.033	1.029	1.027
S.D.	0.009	0.004	0.009	0.010	0.009	0.010
n	26	21	23	25	17	113
Range	1.005 to 1.044	1.023 to 1.041	1.014 to 1.044	1.013 to 1.049	1.017 to 1.045	1.005 to 1.049

Highly significant differences ($p<0.01$) arose between ponies in the specific gravity of their urine samples.

TABLE No.25

pH OF URINE SAMPLES

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	8.35	8.50	8.55	8.60	8.55	8.50
S.D.	0.55	0.50	0.50	0.50	0.50	0.50
n	26	22	23	25	17	113
Range	7.35 to 9.20	7.70 to 9.10	7.85 to 9.00	7.50 to 9.05	7.45 to 9.10	7.35 to 9.20

Differences between ponies with respect to urine pH values were insignificant ($p>0.05$). All ponies exhibited very similar ranges of urine pH values.

TABLE No.26

NET ACID/BASE CONTENT

(mEq/24 hours)

N.B. Net Acid excretion expressed as -ive, Net Base as +ive.

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
Mean	304	336	355	469	438	374
S.D.	272	117	105	158	343	233
n	17	13	14	10	16	70
Range	-102 to 1022	181 to 591	129 to 484	239 to 775	28 to 1396	-102 to 1396

Differences between ponies in the daily excretion of net base were highly significant ($p<0.01$) and the pony who excreted the greatest quantity of base also excreted more urinary inorganic phosphate than the other ponies.

TABLE No.27

SODIUM CONTENT

(mEq/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	84	116	116	127	140	116
S.D.	118	82	46	87	109	92
n	21	20	18	20	17	96
Range	8 to 510	16 to 329	58 to 226	20 to 338	27 to 375	5 to 510

There was no significant difference ($p>0.05$) between ponies in their 24 hour urinary sodium excretion.

TABLE No.28

POTASSIUM CONTENT

(mEq/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	1008	1018	1072	1060	1221	1071
S.D.	399	410	324	406	595	429
n	21	20	18	20	17	96
Range	314 to 1855	265 to 1760	554 to 1788	429 to 2100	529 to 2424	265 to 2424

Differences between ponies in daily urinary potassium excretion were insignificant ($p>0.05$).

TABLE No.29

CHLORIDE CONTENT

(mEq/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	536	551	621	605	774	611
S.D.	294	163	182	341	392	292
n	21	20	18	20	17	96
Range	125 to 1268	282 to 916	237 to 952	235 to 1540	243 to 1539	125 to 1540

The differences between ponies in urinary chloride excretion were significant ($p<0.05$).

TABLE No.30

INORGANIC PHOSPHATE CONTENT

(mg P/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	61.6	147.9	72.7	320.3	72.6	136.4
S.D.	32.7	157.5	38.7	191.3	50.5	151.4
n	26	22	18	21	16	103
Range	9.3 to 159.2	11.6 to 643.1	24.4 to 157.4	89.4 to 920.9	3.2 to 206.9	3.2 to 920.9

One pony (Ben) excreted markedly more urinary inorganic phosphate than the others and hence differences between ponies were highly significant ($p<0.01$). No significant difference between the urinary pH of this pony (Ben) and those of the others was detected, though net base excretion/24 hours by Ben was greater than the daily net base excretion of the other ponies.

TABLE No.31

AMMONIUM CONTENT

(mEq/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	223	192	260	214	338	239
S.D.	115	140	160	84	222	150
n	26	22	18	18	15	99
Range	34 to 514	0 to 577	36 to 581	86 to 437	47 to 680	0 to 680

Differences between ponies in ammonium excretion/24 hours were significant ($p<0.05$). No correlation between ammonium content and net base content, nor between urine pH and ammonium concentration, was evident.

TABLE No.32

UREA CONTENT

(g/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	23.6	30.2	32.8	32.3	42.5	31.9
S.D.	8.9	13.5	12.6	17.9	20.8	15.7
n	19	20	18	17	15	89
Range	10.7 to 46.7	12.9 to 70.5	12.4 to 67.5	17.3 to 82.2	15.8 to 82.0	10.7 to 82.2

Differences between ponies in urinary urea excretion/24 hours were significant ($p<0.05$).

TABLE No.33

*"TOTAL NITROGEN" CONTENT

(g/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	15.4	16.5	19.1	17.7	24.3	15.4
S.D.	5.7	6.1	5.6	7.7	8.0	7.1
n	19	20	18	19	16	92
Range	6.5 to 34.9	6.5 to 33.7	11.6 to 37.1	10.9 to 40.6	13.0 to 40.6	6.5 to 40.6

*The nitrogen from urea and ammonium only.

Differences between ponies in urinary "total nitrogen" excretion/24 hours were significant ($p<0.05$).

TABLE No.34

RESULTS OF ANALYSES OF URINE SAMPLES VOIDED BY BILLIE OVER A 24 HOUR PERIOD

Pony	Billie				
Sample No.	1	2	3	4	Total/24 hours
Time of Voidance (hours)	$4\frac{1}{2}$	6	$9\frac{5}{6}$	$18\frac{1}{3}$	
Volume (ml)	870	280	500	800	2400
pH	8.50	7.90	8.50	8.60	-
S.G.	1.041	1.040	1.047	1.051	-
Na (mEq)	75/1 = 65/S	45/1 = 13/S	30/1 = 15/S	25/1 = 20/S	113
K (mEq)	480/1 = 418/S	470/1 = 132/S	560/1 = 280/S	630/1 = 504/S	1330
Cl (mEq)	182/1 = 158/S	274/1 = 78/S	206/1 = 103/S	188/1 = 150/S	489
NH_4 (mEq)	61/1 = 53/S	31/1 = 11/S	51/1 = 25/S	81/1 = 65/S	154
Urea (g)	13.3/1 = 11.6/S	11.8/1 = 3.3/S	12.4/1 = 6.2/S	12.6/1 = 10.1/S	31.2
Total N_2(g)	6.3/1 = 5.5/S	5.7/1 = 1.6/S	5.8/1 = 2.9/S	6.0/1 = 4.8/S	14.8
Inorg.P.(mg)	25.3/1 = 22.0/S	7.1/1 = 2.1/S	11.4/1 = 5.7/S	22.9/1 = 18.2/S	48

S = quantity/sample

TABLE No.35

RESULTS OF ANALYSES OF URINE SAMPLES VOIDED BY BEN OVER A 24 HOUR PERIOD

Pony	Ben				
Sample No.	1	2	3	4	Total/24 hours
Time of Voidance (hours)	$1\frac{2}{3}$	$6\frac{1}{2}$	$11\frac{5}{6}$	$23\frac{1}{2}$	
Volume (ml)	260	1490	720	940	3410
pH	8.50	8.40	8.50	8.40	
S.G.	1.003	1.015	1.038	1.046	
Na (mEq)	20/1 = 5/S	48/1 = 77/S	25/1 = 17/S	15/1 = 14/S	113
K (mEq)	35/1 = 9/S	130/1 = 194/S	440/1 = 317/S	530/1 = 498/S	1018
Cl (mEq)	16/1 = 4/S	73/1 = 109/S	162/1 = 117/S	193/1 = 181/S	411
NH_4 (mEq)	18/1 = 5/S	41/1 = 61/S	56/1 = 41/S	68/1 = 64/S	172
Urea (g)	1.2/1 = 0.3/S	5.1/1 = 7.6/S	11.0/1 = 7.9/S	11.9/1 = 11.2/S	27.0
Total N_2 (g)	0.4/1 = 0.1/S	2.4/1 = 3.6/S	5.1/1 = 3.7/S	5.6/1 = 5.3/S	12.7
Inorg.P (mg)	52.6/1 = 13.8/S	81.5/1 = 121.4/S	67.9/1 = 48.7/S	66.6/1 = 62.6/S	246.5

S = quantity/sample

TABLE No.36

RESULTS OF ANALYSES OF URINE SAMPLES VOIDED BY BEN OVER A 24 HOUR PERIOD

Pony	Ben				
Sample No.	1	2	3	4	Total/ 24 hours
Time of Voidance (hours)	$2\frac{2}{3}$	$6\frac{1}{2}$	$10\frac{2}{3}$	$22\frac{1}{2}$	
Volume (ml)	1910	670	710	1170	4460
pH	8.60	8.60	8.40	8.40	-
S.G.	1.006	1.025	1.034	1.046	-
Na (mEq)	88/1 = 130/S	113/1 = 76/S	28/1 = 20/S	68/1 = 80/S	306
K (mEq)	35/1 = 67/S	230/1 = 154/S	420/1 = 298/S	45/1 = 53/S	572
Cl (mEq)	26/1 = 50/S	28/1 = 19/S	111/1 = 79/S	266/1 = 311/S	459
NH_4 (mEq)	12/1 = 21/S	40/1 = 27/S	41/1 = 29/S	16/1 = 19/S	96
Urea (g)	1.9/1 = 3.7/S	7.2/1 = 4.8/S	9.7/1 = 6.9/S	1.8/1 = 2.1/S	17.5
Total N_2 (g)	0.9/1 = 1.8/S	3.4/1 = 2.3/S	4.6/1 = 3.3/S	1.2/1 = 1.0/S	8.4
Inorg. P (mg)	42.3/1 = 80.7/S	29.4/1 = 24.3/S	156.7/1 = 111.3/S	258.7/1 = 302.7/S	519.0

S = quantity/sample

TABLE No.37

RESULTS OF ANALYSES OF URINE SAMPLES VOIDED BY SCRUFFY OVER A 24 HOUR PERIOD

Pony	Scruffy				
Sample No.	1	2	3	4	Total/24 hours
Time of Voidance (hours)	Not Obtained				
Volume (ml)	690	300	300	3900	5280
pH	8.20	8.50	8.80	8.10	-
S.G.	1.033	1.036	1.036	1.012	-
Na (mEq)	9/1 = 6/S	12/1 = 4/S	24/1 = 7/S	45/1 = 180/S	197
K (mEq)	350/1 = 424/S	405/1 = 122/S	410/1 = 123/S	120/1 = 479/S	966
Cl (mEq)	166/1 = 115/S	119/1 = 36/S	70/1 = 21/S	36/1 = 144/S	316
NH_4 (mEq)	97/1 = 67/S	65/1 = 19/S	80/1 = 24/S	53/1 = 212/S	322
Urea (g)	18.1/1 = 12.5/S	20.3/1 = 6.1/S	4.0/1 = 1.2/S	7.2/1 = 28.1/S	47.9
Total N_2 (g)	8.4/1 = 5.9/S	9.7/1 = 2.9/S	2.0/1 = 0.6/S	3.4/1 = 13.4/S	22.8
Inorg.P (mg)	52.8/1 = 36.5/S	51.9/1 = 15.7/S	29.8/1 = 8.9/S	10.3/1 = 41.5/S	102.6

S = quantity/sample

DISCUSSION

The volumes of urine voided by these ponies over a twenty-four hour period were not likely to have been identical with the volumes of urine formed over this time, since the volume voided depended upon the times the ponies emptied their bladders. If a pony commenced the collecting period with a full bladder which he emptied soon after being put into harness, and if at the end of the collecting period the bladder was emptied again, the volume of urine collected was likely to be greater than that formed by the kidneys over twenty-four hours. Conversely, if a pony commenced the collecting period with an empty bladder, and ended it with a full one, the volume of urine collected would then be less than that formed over twenty-four hours. To minimise the differences between the volumes voided/24 hours and the volumes formed/ 24 hours a large number of samples was collected which should have yielded values approximating closely with the mean urine volume formed over this period of time. Alternatively, a more accurate approach would have involved catheterising the ponies, but this was decided against.

In view of the large number of urine samples required from the ponies it was believed that repeated catheterisation would have incurred a considerable risk of urinary tract infection. Furthermore, because blood samples were frequently collected at these times, the packed cell volume percentages might have been increased, due to apprehension caused by catheterisation.

The possibility that the concentrations of the constituents under investigation in urine voided during a single micturition might be identical, or nearly identical, with the concentrations in the whole twenty-four hour volume was explored. The result from three ponies who were investigated are presented in Tables No. 34 to 37. The results clearly indicate that the volume, pH, specific gravity and the concentrations of constituents studied varied considerably between samples. Only results from ponies who voided urine four times during the twenty-four hours they were observed are presented. Other ponies were initially included in the investigation, but were excluded when they voided urine more than once overnight, thus preventing samples being collected individually. In all ponies studied micturition was infrequent, and occurred 3 to 6 times daily.

It was concluded that complete twenty-four hour collections were necessary for the accurate determination of the urine parameters investigated. It was not possible to estimate the quantities of these constituents lost/24 hours simply by measuring their concentrations in a single sample, and then calculating excretion/24 hours on this basis.

The volumes of water imbibed were not measured. The mean urine volumes/24 hours voided by these ponies were similar to those reported by Nicholson[89]. This was expected, since four of the five ponies studied in the course of this work were also included in the group of five

ponies investigated by Nicholson[89]. The reasons for the greater volume of urine voided/kg body weight/day by Scruffy are unknown.

Compared with the results obtained from other animals by other workers[5 85 86] the urine volumes/kg body weight/24 hours voided by these ponies are high. Whilst no reasons were ascertained, the conditions under which the urine collections took place may have had some bearing upon the volumes obtained. The Shetland and Shetland-cross ponies were subjected to a cool environment, and they were not exercised, so under this system of management it is probable that sweating was minimal. The horses of other workers were managed under warmer climatic conditions, were sometimes exposed to direct sunlight, and were sometimes afforded facilities for exercise, so that water lost in sweat may have detracted from that in urine. The five ponies studied in the course of this work were well accustomed to the urine collecting apparatus, but it has been observed that until a pony becomes accustomed to the procedure of urine collection voluntary retention of urine occurs. This might have been the cause of the low volumes obtained by others[5 85 86].

The specific gravity values of the urine of these ponies are lower than those published by Ellenberger and Scheunert[5 26]. It is likely that the lower specific gravity values observed in Scruffy caused the difference between ponies to be highly significant.

A marked trend towards a reciprocal relationship between the volume and the specific gravity of urine from

these ponies was noted. Hence, since the ponies voided a greater volume of urine/kg body weight than the horses observed by others[5 26 85 86], lower specific gravity values would be expected. Although urine volume and specific gravity exhibited a degree of reciprocity the relationship between these two parameters was not simple[89].

All the individual mean urine pH values of these ponies were greater than those observed by Nicholson[89], and all were above the upper limit of the range discovered by Ellenberger and Scheunert, quoted by Tasker[26]. It was not known why these ponies produced a more alkaline urine than other equines studied. In no twenty-four hour urine sample collected was the pH acid[91]. Urine pH was considered acid when it was less than the pH of blood. Though one pony (Scruffy) produced a sample of pH 7.35 this was accepted as neutral.

Nicholson[89] stated that the pH of urine was merely a guide to the hydrogen ion concentration owing to the effects exerted by other ions present. The net acid/base content of urine[95] was considered to be a more realistic indication of the true acid/base status of the urine, because it eliminated the influence upon pH of these ions. The bicarbonate content of the urine samples was not measured separately in the course of this work because the samples were exposed to the atmosphere for periods of up to twenty-four hours. Had a protective layer of mineral oil been used, practical difficulties with measurements of volume, pH, and other analyses would have been introduced.

In addition the possibility that bicarbonate and/or carbon dioxide could have entered the mineral oil would have existed[84]. Only one pony (Scruffy) excreted net acid. This occurred on two occasions, despite the fact that the pH of the samples was alkaline on each occasion. No obvious relationship between urine pH and acid-base content or concentration was observed.

In terms of mEq/24 hours, the mean daily urinary sodium excretion of these ponies was similar to that observed by Nicholson[89], but owing chiefly to discrepancies in the estimated daily sodium intake the percentage of the estimated sodium intake voided in urine differed markedly from the value quoted by Nicholson[89]. From the results of hay analyses[97] it was estimated that the ponies ingested 313 mEq of sodium daily of which a mean of 116 mEq was excreted in urine. This represents 37% of that ingested. Nicholson[89] estimated that 54% of the daily sodium intake was lost in urine. However, since the composition of hay varies considerably[97] and because only two samples of hay were analysed the results obtained can only be accepted as a general guide. Nevertheless, the results obtained from these ponies grossly exceeded those values observed by Tasker[86]. Analyses of hay samples indicated that the daily intake of potassium by the ponies was 2026 mEq, and a mean urinary loss of 1071 mEq represents 52% of the intake. This compares closely with Tasker's[86] result, which showed that out of 3930 mEq of potassium ingested, 2169 mEq - or 55% - was lost in urine.

Nicholson's[89] estimate of 99% urinary excretion of the potassium intake appears extraordinarily high, in view of the loss of potassium in the faecal fluids, which will be described at a later stage.

From his study of the intake and output of sodium and potassium Tasker[86] concluded that the sodium intake of horses fed exclusively on hay was extremely low, and that a strict sodium conservation mechanism must maintain the animals in sodium balance. His study also revealed the intake of very large amounts of potassium and it was apparent to him that the excretion of potentially highly toxic quantities of potassium was a vitally important renal function.

Daily chloride intake, calculated from the results of hay analyses, was 812 mEq/24 hours and mean urinary chloride excretion was 611 mEq/24 hours, i.e. 75% of that ingested. Investigations of chloride intake and loss were outwith the scope of the work of others, and so no comparisons were possible.

No reference to urinary inorganic phosphate excretion by equine animals was discovered, so no comparisons with other horses could be made. In no pony studied was there any correlation between urinary pH and inorganic phosphate concentration nor between net acid-base content and inorganic phosphate content.

It was not known why Ben excreted more urinary inorganic phosphate than the other four ponies. However, the fact that this occurred could have been the cause of

the greater base excretion by this pony[95], since the majority of the inorganic phosphate would be in the form of the monohydrogen phosphate[105]. Generally, the greater the pH of urine, the greater will be the proportion of inorganic phosphate present as monohydrogen phosphate. However, due to the activity of other ions present in the urine, the proportions of inorganic phosphate present in the mono and dihydrogen forms cannot be accurately deduced from the pH of the urine sample and the pK_2 of phosphate[180 181]. It was therefore decided to express the inorganic phosphate content of the urine samples analysed as mg of phosphorus.

Although the ammonium content of urine voided by these ponies was measured, interpretation of the results obtained was difficult. The urine collecting bottles were placed in a cool well but it was unlikely that this would have completely prevented urea-splitting and the consequent *in vitro* formation of ammonium. It was discovered that even after storage at 4°C the ammonium content of an aliquot of urine increased slightly.

The addition of a preservative such as toluene[98] to the collecting bottle might have prevented urea splitting, but because many other analyses were performed upon the urine collected, and because the effects of toluene upon such analyses were unknown, the use of this preservative was decided against. Each gram of urea contains 467 mg of nitrogen, and from this 33 mEq of ammonium can be formed. Thus, any urea splitting would have caused a decrease in

the urinary urea content simultaneously with an increase in urinary ammonium.

Since the hydrolysis of urea and consequent ammonium production was considered a likely source or error, it was decided to measure the so-called "total nitrogen" content of the urine samples. This is the nitrogen derived from urea and ammonium only. The quantity found present in a urine sample is not affected by the degree of urea hydrolysis since the accuracy of the result obtained depends upon the complete hydrolysis by urease of all the urea in the aliquot analysed. The nitrogen derived from both ammonium and urea is then measured as a single entity. Although there were significant difference ($p<0.05$) between ponies in total nitrogen excretion/24 hours, total nitrogen excretion/kg body weight/24 hours ranged from 0.09 to 0.10 g and thus appeared related to body weight.

Unfortunately no record by other workers of urinary ammonium and urea excretion in horses or ponies was discovered, and therefore it was not possible to compare the results from these ponies with any others.

PART 3

FAECAL FLUID

INTRODUCTION

Of the various aspects of water and electrolyte studies in equines, the fluids and electrolytes of the gut and faeces appear to have attracted the least attention, though Tasker[86 87 88 92], Alexander[23 55 99 100 101] and Comline, Hall, Hickson, Murillo and Walker[192] have investigated the rôle of the equine gut and the associated exocrine glands in water and electrolyte balance.

Tasker[86] investigated the faecal output of four horses whose weights ranged from 411 to 485 kg. These horses consumed 10 kg hay daily. The mean volume of drinking water imbibed daily was 23.6ℓ and Tasker[86] calculated that a further 1.1ℓ/day was obtained from hay, giving a mean total daily water intake of 24.7ℓ/day or 54 ml/kg body weight. The mean daily weight of faeces produced was 18 kg and this contained 14.0ℓ of water (56.7% of the total daily intake), c.f. 4.9ℓ/day (19.8% of the total daily intake) in urine.

Of the electrolytes present in faecal fluids, Tasker[86] investigated only sodium and potassium. The mean daily faecal sodium loss in his horses was 116 mEq, or 35.3% of the intake, and mean daily faecal potassium loss was 993 mEq, or 25.3% of the intake.

Tasker[87 92] found that the loss of faecal water and sodium was greatly increased when scouring occurred, though the output of faecal potassium was only slightly

elevated. Withholding food and water was observed to reduce both the quantity of faeces passed and the water content of the faeces[88]. Tasker[88] quoted Sisson and Grossman's[93] estimate that the digestive tract of the horse normally contains circa 100ℓ of water, much of which is believed to be available for absorption.

Alexander[23 55 99 100 101] studied many aspects of the physiology, biochemistry and pharmacology of the equine digestive tract very extensively. Though no record of faecal analyses was discovered, much of his work, especially his studies of the intestinal regions of the gut, has a direct bearing upon the results of the investigations of faecal fluid about to be described. Therefore a short description of the composition of some digestive tract fluids is relevant here.

Alexander[23] measured the pH and various constituents of the digestive tract fluids of horses immediately after slaughter. He observed that only in the caudal ileum was the mean pH value alkaline, and only in the stomach did the pH deviate widely from near neutrality. Mean sodium concentrations were lower than the normal range of plasma sodium levels in the stomach, jejunum and cranial ileum, approximately equivalent to plasma levels in the caudal ileum and caecum, and lower than the plasma levels in the dorsal and ventral segments of the large colon, and in the small colon. Alexander[23] considered that the decrease in sodium concentration in the regions of the gut distal to the caecum was probably an active conservation mechanism because the ions were apparently moving against a

concentration gradient from the colon to the plasma.

Potassium concentrations decreased progressively from the stomach to the caudal ileum and then increased progressively from the caecum to the small colon where potassium concentration was greatest. Throughout the gut the potassium concentration in the various fluids was many times greater than the usual plasma levels. Alexander[23] stated that since the major part of the gut fluid reabsorption occurs in the dorsal and small colon, it seemed not unreasonable to believe that the increase in potassium concentration in these regions of the gut could be partly due to water reabsorption unaccompanied by potassium reabsorption.

Chloride concentration in the stomach was found to be high[23], and salivary chloride is likely to contribute to the content in the stomach, in addition to gastric hydrochloric acid secretion[55 189]. The mean concentration of chloride decreased from the stomach to the jejunum increased in the cranial ileum and then decreased throughout the remainder of the tract. It was discovered that equine pancreatic fluid is rich in sodium and chloride ions[102 192] and this undoubtedly accounted for part of the increased chloride concentration in the proximal regions of the small intestine.

Concurrent with the decrease in chloride concentration which was observed in those regions of the gut beyond the cranial ileum[23] was a marked rise in bicarbonate concentration, and Alexander[23] cited evidence in support of

this increase being probably mainly due to the secretion of bicarbonate, from the serosal to the mucosal sides of the intestine.

In the dorsal colon inorganic phosphate doubled in concentration from that in the ventral colon and replaced bicarbonate as the major buffer, though bicarbonate was still present[23]. No explanation was offered for this discovery, and it does not appear to be established why there should be a change in the predominance of different buffers in different regions of the lower gut. In the small colon inorganic phosphate concentration was also much higher than that of bicarbonate, though the levels of both phosphate and bicarbonate decreased somewhat.

Alexander[101] stated that the concentration of urea is higher in the colon than elsewhere in the digestive tract, and he postulated that endogenous urea might pass from the gut wall into the lumen. Alexander and Davies[103] found urea throughout the digestive trace from the stomach to the small colon. The highest concentrations were those in the large colon, though high concentrations were present in the small colon too. Using paired intestinal content samples from a pony with indwelling cannulae in the ventral and dorsal segments of the large colon, Davies[104] was able to demonstrate urea concentrations in the dorsal colon that were approximately twice those in the ventral colon.

A quantitative study of the faecal fluids excreted by the five Shetland and Shetland-cross ponies was undertaken for three reasons:-

1) To assess the output of sodium, potassium and chloride in faeces, in an effort to balance the intake and output of these electrolytes.
2) To assess the relative importance of the faecal and urinary routes of excretion of selected electrolytes and urea.
3) To compare the composition of digestive tract fluids, especially small colon fluid, with faecal fluid.

METHODS

THE COLLECTION OF 24 HOUR FAECES SAMPLES FROM UNTREATED PONIES, AND THE ANALYSIS OF FAECAL FLUID

Faeces were collected over twenty-four hour periods from the same five Shetland and Shetland-cross ponies from whom blood and urine samples were obtained. Diet and management have already been described, though the collection of faeces necessitated the ponies standing in stalls for the duration of the collection.

The collecting apparatus is illustrated in the photograph overleaf. The plastic shute was attached at its proximal end to a small rubber-covered triangular metal frame which fitted around the anus of the pony. The shute extended from immediately below the anus to approximately six inches above the base of the large plastic collecting bin. The frame was secured in situ by three straps. The forked upper strap formed a crupper which was secured around the webbing girth. Each of the lateral straps passed from the lateral corners of the triangular frame behind and between the animal's hind legs and extended forwards along the flanks. These were also secured around the girth. The harness was fitted sufficiently tightly to prevent the shute slipping, or being pulled up and away from beneath the anus when the pony lifted his tail immediately before defaecation. As an additional safeguard against loss of a faecal sample the shute was surrounded by a thin polythene funnel. This extended at its upper end from beneath the shute to above the pony's tail, and

APPARATUS FOR THE COLLECTION OF FAECES FROM PONIES

at its lower end it encircled the collecting bin to which the sides of the shute were secured with clamps. When the pony stood in a stall two horizontal metal bars were interposed between the rear of the pony and the collecting bin to prevent the bin being kicked over or away.

The collecting bin was weighed before collection commenced, and again immediately after collection. The difference between the two weights represented the weight of faeces voided over the preceding 24 hours. The faeces were thoroughly mixed before sampling, and a weighed aliquot was dried at 110°C to constant weight. This required 24 to 28 hours, and the difference in weight before and after drying represented the water content of the faecal sample. The percentage of water in the faeces was then calculated, and from the result obtained the total volume of water lost in the faeces passed over a 24 hour period was also calculated.

Fluid was expressed from a portion of the remaining well mixed faeces, and pH, electrolyte, urea and net acid/base determinations were made upon this fluid. pH was measured to 0.05 pH unit with an AML Model 40 pH meter. Sodium and potassium concentrations were measured by flame photometry, using an EEL flame photometer as described in Part 1 of this section. Dilutions of faecal fluid for sodium and potassium determinations were 1:50 and 1:500 respectively. Chloride concentration was measured using an EEL chloride meter, and 10 ml aliquots of a 1:50 dilution of faecal fluid. Inorganic phosphate was determined by the method of Delsal and Manhouri, described by Wootton[29]. It was found necessary to dilute the faecal fluid 1:50 before these

determinations were commenced. Ammonium, urea and "total nitrogen" concentrations were measured by Nessler's method, also described by Wootton[29]. Net acid/base concentration was determined by the modification of Jørgensen's method[95] which has already been fully described in Part 2.

From the knowledge of the concentrations in faecal fluid of electrolytes, urea and net acid/base the excretion/24 hour was calculated. The total mean daily quantities/kg body weight of sodium, potassium, chloride, ammonium, urea, "total nitrogen", inorganic phosphate and net acid/base excreted in the urine and faecal fluids of each pony were also calculated.

RESULTS

Summaries of the quantities of faeces voided/24 hours, the weight of faeces/kg body weight/24 hours, the water content and the faecal water lost/kg body weight/24 hours, the pH and the quantities of urea and selected electrolytes in faecal fluid are presented in Tables 38 to 51. The results were subjected to analysis of variance in order to investigate whether differences between ponies were statistically significant.

Complete lists of individual results are presented in Appendix No.3. The mean daily quantities/kg body weight of sodium, potassium, chloride, ammonium, urea, total nitrogen, inorganic phosphate and net acid/base excreted in the urine and faecal fluids of the ponies are listed in Table No. 52.

TABLE No.38

WEIGHT OF FAECES PASSED IN 24 HOURS

(kg)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	4.310	3.595	4.465	4.825	5.840	4.610
S.D.	1.030	1.060	0.770	1.000	0.905	1.155
n	6	6	6	6	6	30
Range	2.730 to 5.635	2.210 to 5.075	3.225 to 5.305	3.250 to 5.780	4.830 to 7.170	2.210 to 7.170

TABLE No.39

WEIGHT OF FAECES PASSED/kg BODY WEIGHT/24 HOURS

(g)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	24.1	21.9	24.6	26.6	27.3	24.9
S.D.	5.8	6.5	4.2	5.5	4.2	6.2
n	6	6	6	6	6	30
Range	15.3 to 31.5	13.5 to 30.9	17.8 to 29.2	17.9 to 31.9	22.6 to 33.5	13.5 to 33.5

Despite the ponies receiving identical diets (with the exception of Scruffy, who, in addition to his hay, received a small oats/bran supplement containing 20 g of sodium bicarbonate[55]), the largest pony voided the greatest weight of faeces/24 hours and the smallest pony the smallest quantity of faeces/24 hours. This relationship still holds if the units are expressed as g/kg body weight/24 hours though the differences between ponies in faeces passed/kg body weight/24 hours are not statistically significant ($p>0.05$).

TABLE No.40

% WATER CONTENT OF FAECES

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	80	76	80	78	79	79
S.D.	1	3	2	3	1	3
n	6	6	6	6	6	30
Range	79 to 82	72 to 78	79 to 83	74 to 81	77 to 80	72 to 83

TABLE No.41

WATER CONTENT OF FAECES PASSED IN 24 HOURS

(ml)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	3450	2740	3590	3770	4580	3620
S.D.	850	880	600	740	720	930
n	6	6	6	6	6	30
Range	2180 to 4510	1590 to 3910	2610 to 4190	2400 to 4440	3770 to 5660	1590 to 5660

TABLE No.42

WATER CONTENT OF FAECES/kg BODY WEIGHT/DAY

(ml)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	19.3	16.7	19.8	20.8	21.4	19.5
S.D.	4.8	5.4	3.3	4.1	3.4	5.0
n	6	6	6	6	6	30
Range	12.2 to 25.2	9.7 to 23.8	14.4 to 23.1	13.5 to 24.5	17.6 to 26.4	9.7 to 26.4

On the basis of volumes of faecal fluid/kg body weight/24 hours the volumes of faecal water lost did not differ significantly between ponies ($p>0.05$).

TABLE No.43

pH OF FAECAL FLUID

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	6.65	6.05	6.25	6.45	6.25	6.35
S.D.	0.30	0.25	0.25	0.15	0.10	0.30
n	6	6	6	6	6	30
Range	6.25 to 7.15	5.85 to 6.50	5.95 to 6.70	6.25 to 6.60	6.15 to 6.35	5.85 to 7.15

TABLE No.44

*NET ACID/BASE CONTENT OF FAECAL FLUID

(mEq/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	28	-3	-35	14	9	3
S.D.	36	88	38	32	31	51
n	6	6	6	6	6	30
Range	-31 to 66	-75 to 170	-96 to 2	-33 to 46	-29 to 64	-96 to 170

* Net acid expressed as -ive
Net base expressed as +ive

No significant differences between ponies in faecal fluid pH was observed ($p>0.05$), but significant differences between ponies in the net acid/base content of the faecal fluid occurred ($p<0.05$).

TABLE No.45

SODIUM CONTENT OF FAECAL FLUID

(mEq/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	22	44	66	50	72	51
S.D.	11	21	36	35	47	35
n	6	6	6	6	6	30
Range	10 to 41	18 to 82	32 to 118	23 to 114	39 to 139	10 to 139

Differences between ponies in daily sodium loss in faecal fluids were significant ($p<0.05$).

TABLE No.46

POTASSIUM CONTENT OF FAECAL FLUID

(mEq/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	263	174	264	290	358	269
S.D.	102	78	88	86	81	101
n	6	6	6	6	6	30
Range	158 to 400	87 to 283	178 to 398	177 to 444	236 to 489	87 to 489

Highly significant differences in daily potassium loss in faecal fluids were observed between ponies ($p<0.01$).

TABLE No.47

CHLORIDE CONTENT OF FAECAL FLUID

(mEq/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	20	11	15	37	21	21
S.D.	15	11	6	65	13	30
n	6	6	6	6	6	30
Range	10 to 39	0 to 33	8 to 23	4 to 169	8 to 45	0 to 169

Significant differences in the chloride content of the faecal fluids of these ponies were evident ($p<0.05$).

TABLE No.48

AMMONIUM CONTENT OF FAECAL FLUID

(mEq/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	30	43	65	46	49	47
S.D.	30	31	14	36	29	29
n	6	6	6	6	6	30
Range	4 to 76	5 to 97	39 to 81	9 to 102	26 to 100	4 to 102

Differences between ponies in the ammonium content of faecal fluid were highly significant ($p<0.01$).

TABLE No.49

UREA CONTENT OF FAECAL FLUID

(g/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	1.5	2.2	2.1	1.7	4.4	2.4
S.D.	2.1	1.9	1.5	2.2	5.4	2.9
n	6	6	6	6	6	30
Range	0.3 to 5.7	0.4 to 5.2	0.7 to 4.5	0.1 to 6.1	0.1 to 14.7	0.1 to 14.7

TABLE No.50

*TOTAL NITROGEN CONTENT OF FAECAL FLUID

(g/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	1.1	1.6	1.9	1.4	2.6	1.7
S.D.	1.3	1.3	0.8	1.3	2.9	1.6
n	6	6	6	6	6	30
Range	0.3 to 3.7	0.3 to 3.7	0.9 to 3.2	0.2 to 3.8	0.4 to 7.8	0.3 to 7.8

*The nitrogen content of urea and ammonium only.

Differences between ponies in the urea and "total nitrogen" contents of faecal fluid were significant ($p<0.05$).

TABLE No.51

INORGANIC PHOSPHATE CONTENT OF FAECAL FLUID

(mgP/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
$\bar{x}$	3464	2352	5129	3039	4861	3731
S.D.	1491	1697	1609	1254	2412	1912
n	6	6	6	6	5	29
Range	1383 to 4978	939 to 5558	3346 to 7714	1260 to 5061	1373 to 7721	939 to 7721

Differences between ponies in the inorganic phosphate content of the faecal fluids were highly significant ($p<0.01$) and the differences were not solely due to weight variations between ponies.

TABLE No.52

MEAN DAILY LOSSES OF SELECTED CONSTITUENTS OF THE URINE AND FAECAL FLUID/kg BODY WEIGHT OF PONIES

Pony		Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
Body Weight (kg)		178.7	164.2	181.4	181.4	214.1	184.0
Na mEq	Urine	0.47	0.71	0.64	0.70	0.65	0.63
	Faecal Fluid	0.12	0.27	0.36	0.28	0.34	0.28
	Total	0.59	0.98	1.00	0.98	0.99	0.91
K mEq	Urine	5.64	6.20	5.91	5.84	5.70	5.82
	Faecal Fluid	1.47	1.06	1.46	1.60	1.67	1.46
	Total	7.11	7.26	7.37	7.44	7.37	7.28
Cl mEq	Urine	3.00	3.36	3.42	3.34	3.61	3.32
	Faecal Fluid	0.11	0.07	0.08	0.20	0.10	0.11
	Total	3.11	3.43	3.50	3.54	3.71	3.43
Inorganic P mg	Urine	0.3	0.9	0.4	1.8	0.3	0.7
	Faecal Fluid	19.4	14.3	28.3	16.8	22.7	20.3
	Total	19.7	15.2	28.7	18.6	23.0	21.0
NH_4 mEq	Urine	1.25	1.17	1.43	1.18	1.58	1.30
	Faecal Fluid	0.17	0.26	0.36	0.25	0.23	0.26
	Total	1.42	1.43	1.79	1.43	1.81	1.56
Urea mg	Urine	132	184	181	178	199	173
	Faecal Fluid	8	13	12	9	21	13
	Total	140	197	193	187	220	186

(Contd.

TABLE No.52 Continued

Pony		Scruffy	Jimmie	Billie	Ben	MacGowan	All Ponies
"Total" N_2 [+] mg	Urine	86	101	105	98	114	100
	Faecal Fluid	6	9	11	8	12	13
	Total	92	110	116	106	126	113
Net Acid/Base [*] mEq	Urine	1.70	2.05	1.96	2.59	2.05	2.03
	Faecal Fluid	0.16	-0.02	-0.19	0.08	0.04	0.02
	Total	1.86	2.03	1.77	2.67	2.09	2.05

[+] The nitrogen derived from ammonium and urea.

[*] Net acid expressed as -

With the exclusion of Scruffy, mean daily sodium loss/kg body weight was almost identical. Mean daily potassium losses/kg body weight were reasonably similar in all ponies, as were chloride losses. Marked differences between ponies ($p<0.05$) occurred in mean daily inorganic phosphate loss/kg body weight. Significant differences ($p<0.05$) between ponies were also observed in the mean daily excretion/kg body weight of ammonium, urea, "total nitrogen" and net base.

DISCUSSION

The quantity of faeces collected over 24 hours represented the quantity excreted rather than the quantity formed, and depended upon the times and the frequency of defaecation.

Tasker's[86] horses, whose weights were circa 2 to 3 times greater than the weights of these ponies, passed approximately 4 times the quantity of faeces produced by the Shetland and Shetland-cross ponies. The mean percentage water content of the faeces produced by the horses was 74%, compared with a 79% mean faecal water content of the ponies. The water content of the hay fed to the ponies amounted to 12%, so that far more water was lost in the faeces than was ingested in the hay alone. Tasker's[86] horses excreted a greater quantity of dry faecal material/kg body weight/day than did the ponies. They also ingested a slightly greater quantity of dry matter/kg body weight/day.

The mean daily faecal water loss was only approximately half a litre less than the mean daily urine volume voided, (see Section No.1, Part 2) and two of the ponies exhibited an individual mean daily faecal water loss greater than the mean daily urine volume voided. Since equine urine contains solid material the volume of urine is greater than the volume of urinary water, and so it was concluded that in all these ponies the daily volume of water lost in faeces was very similar to that lost in urine.

The ponies differed substantially from Tasker's[86] horses, who lost approximately three times more water in

faeces than in urine. The reasons for this are not evident, but diet was excluded as a major cause of the difference. The horses and ponies received a very similar quantity of hay on a body weight basis. The water content of the hay fed to the horses was 11% and that fed to the ponies 12%, and so small a difference would not account for the substantial discrepancy observed between the horses and the ponies in the faecal water loss. However, the mean water loss/kg body weight in the urine and faeces of Tasker's horses[86] is similar to that of the Shetland ponies i.e. 42.9 ml/kg body weight/24 hours and 41.8 ml/kg body weight/24 hours respectively. (Losses via the skin and lungs were not investigated.) Thus it is evident that the differences in water loss between these two groups of equines lies in the route of loss and not in the quantities lost. The reason for this difference is not clear.

No reference to the pH of equine faecal fluid was discovered, although Alexander[23] noted that the mean pH of the liquor in the small colons of eight newly slaughtered horses was 6.50, which resembles that of the faecal fluids of the five ponies studied. Though the pH of all the samples of faecal fluid from these ponies was acid (i.e. below pH 7.400), measurements of the net acid/base content revealed that net base excretion sometimes occurred despite the acid pH of the fluid. It was thus concluded that the presence of substances other than hydrogen ions exerted a strong influence upon the pH of

the faecal fluid[89]. Because the mean net acid/base content of the faecal fluid samples examined was small, compared with that of urine, it was concluded that in these ponies in the untreated state, a reasonably accurate indication of net acid-base excretion could be gained from urine analysis only. The fistulated pony was excluded from this conclusion because the quantity of acid or base lost in saliva from the fistulated parotid duct was not determined.

The fistulated pony (Scruffy) excreted less sodium in both urine and faecal fluid than the others, probably due to sodium lost in saliva[55]. Because all the ponies except the fistulated pony excreted similar quantities of sodium/kg body weight/24 hours in urine and faecal fluid it was concluded that daily sodium excretion was related to body weight when the animals received identical diets.

The overall mean sodium loss/24 hours in faecal fluid is equivalent to 16% of the calculated intake. In this respect these ponies again differ from Tasker's[86] horses, whose daily faecal sodium loss represented 35.3% of the intake. Urinary and faecal sodium losses in Tasker's[86] horses together accounted for 37.4% of the daily ingested sodium. Hence the general pattern of sodium excretion and water excretion by Tasker's horses differed markedly to that observed in the ponies. In the former group of animals faecal sodium loss was many times greater than urinary sodium loss. In the latter group of equines, the mean urinary sodium loss amounted to more than twice the loss in

faecal fluid. The combined daily urinary and faecal sodium losses of the ponies amounted to 53%. Thus 47% of the calculated daily sodium intake of these ponies could not be accounted for. This discrepancy represented 146 mEq of sodium.

Although Tasker[86] was unable to account for 62.6% of the sodium ingested by his horses he observed no evidence of sodium retention. He thus concluded that this sodium was lost in "insensible sweat". It is difficult to accept that nearly half the sodium ingested by the Shetland and Shetland-cross ponies could be excreted in sweat. Though cutaneous water vapour loss no doubt occurred, insensible sweating[105] is the loss of water vapour alone. Sensible sweating, which involves the excretion of water, electrolytes and metabolites[105], was not observed in these ponies during the course of this work.

The mean sodium concentration in the faecal fluids of the ponies was markedly lower than the mean sodium concentration in the liquor of the small colon of newly slaughtered horses examined by Alexander[23]. It was concluded that there may have been a substantial difference between these ponies and the horses before the latter's death, or sodium might pass into the lumen of the small colon post mortem, or that sodium could be absorbed at a site in the gut distal to the mid small colon. The ponies were similar to Tasker's[86] horses in so far as they excreted a greater quantity of potassium in urine than in faeces, but

the percentage of the daily potassium intake lost in the faecal fluids of the ponies was 13%, whereas 25% of the potassium ingested by Tasker's[86] horses was found to be excreted in their faeces. Thus Tasker[86] was able to account for 80% of the potassium his horses ingested in terms of urinary and faecal losses. He postulated that the remainder must be lost in sweat.

A mean total of 65% of the calculated potassium intake of the ponies was present in their urine and faecal fluids. The 35% unaccounted for amounts to 686 mEq of potassium/24 hours. For reasons already stated it is difficult to accept that this quantity of potassium was excreted in sweat[86]. Soliman and Nadim[82] found that the potassium content of the sweat of heavily exercised stallions was 47.8 mEq/l. Assuming that the sweat produced by these ponies would have an identical potassium concentration, this would necessitate their losing 14.3 litres of water/24 hours via the sweat glands, and sensible sweating was never observed.

Nicholson[89] estimated that only 1% of the potassium ingested by his ponies was lost by routes other than via the kidneys. However, it is suspected that he underestimated daily potassium ingestion.

It was noted that the mean potassium concentration in the liquor of the small colon of the horses subjected to post-mortem examination by Alexander[23] did not differ greatly from the mean faecal fluid potassium concentration of these ponies.

Faecal chloride losses amount to a small proportion of

total daily chloride loss. Only 3% of the calculated quantity of chloride ingested/24 hours was recovered in faecal fluid, compared with 75% in urine. Of the calculated daily chloride intake of these ponies 22%, or 180 mEq, could not be accounted for. Although losses in saliva would have occurred from the fistulated pony, this does not explain the discrepancy also observed in the other four animals. The mean concentration of chloride in the faecal fluids of the ponies was similar to that discovered in the liquor of the small colons of freshly slaughtered horses[23] but no reference to the actual faecal fluid chloride content of horses was discovered. It was concluded that daily urinary and faecal potassium and chloride losses were related to the body weight of these ponies.

It was likely that much of the ammonium in faecal fluid arose from the *in vivo* and *in vitro* hydrolysis of urea. Davies[104] discovered urea-splitting bacteria in both the ventral and dorsal colon of a fistulated pony. Alexander[101] postulated that endogenous urea might pass from the gut wall into the gut lumen. As has already been stated, urea diffuses throughout the cellular and extracellular water of the body[29]. It is obvious, from the urea content of urine and faecal fluids, that the major route of urea excretion is renal. However, in view of the free diffusion of urea throughout the body it appears likely that the gastro-intestinal water forms another fluid "compartment" into which urea is able to diffuse, despite the fact that the contents of the gut are outwith the body itself.

Urea has been discovered throughout the gastro-

intestinal tract from the stomach to the large colon[103], and the concentrations in the colon were found to be the highest throughout the gut[101 104]. Thus there would be no lack of substrate, upon which the aforementioned bacteria could act. Furthermore, it is possible that the urea excreted in faecal fluid could be hydrolysed *in vitro* by bacterial action, consequently giving rise to more ammonium.

The interpretation of the observations of the excretion of the nitrogenous compounds is difficult owing to their interrelationship. The ponies varied with respect to daily ammonium excretion/kg body weight but since ammonium can be produced both *in vivo*, and *in vitro*[53 98 104 105 106] the results obtained are not likely to be a very accurate reflection of ammonium excretion. If any ammonium is produced from urea *in vivo* then the quantities of urea initially present diminishes correspondingly, and this could be another factor which influenced the variations in urea excretion/kg body weight/24 hours.

The discovery of very large quantities of inorganic phosphate in faecal fluid is consistent with the discovery by Alexander[23] of high inorganic phosphate concentrations in the colon liquor of freshly slaughtered horses. Exactly why these large quantities of phosphate are secreted into the colon does not appear to have been established, though Alexander[99 100] suggested that the function was principally that of buffering the volatile fatty acids produced.

Though the urinary inorganic phosphate content of one

pony (Ben) was much greater than that of the others (see Part 2 of this section), the same was not true of his faecal fluid inorganic phosphate. In all ponies faecal inorganic phosphate excretion was far greater than renal excretion. No direct relationships between faecal fluid pH and the inorganic phosphate content, and the net acid/base and inorganic phosphate contents of faecal fluids were evident, though the concentrations of other buffers in the gut might also influence inorganic phosphate concentration.

The very large variations in the mean daily inorganic phosphate excretion/kg body weight are difficult to understand, and without further investigation it is impossible to determine the reasons for this finding. Although inorganic phosphate is present in saliva[55] and hence would be lost via this route from the fistulated pony, the lowest mean daily loss/kg body weight occurred from a non-fistulated pony. Different rates of inorganic phosphate turnover would not be expected to influence these results provided no imbalance occurred. However, since only the inorganic form of phosphorus was investigated it might be that reciprocal variations in organic phosphorus excretion render mean daily total phosphorus excretion/kg body weight identical in all ponies.

SUMMARY OF SECTION No.1

Measurements of the packed cell volume percentages, blood pH and pCO_2 serum bicarbonate concentrations, and the concentrations in plasma of sodium, potassium, chloride, inorganic phosphate and urea were made upon venous blood from five Shetland and Shetland-cross ponies. The specific gravity of blood and plasma was measured. Ranges of normal values for these parameters in these ponies were established, and considerable variations were observed both within and between individual ponies.

The results obtained were compared with data published by others. Similarities were noted in the packed cell volume percentages of the five ponies investigated and those of other Shetland-type animals and draught horses.

The plasma sodium concentrations of the ponies investigated appeared to be lower than those of most other equine animals, and the possibility of this being a genetic trait in Shetland ponies is discussed.

The volumes of urine produced by the ponies over a 24 hour period were measured, and large day to day variations were observed. The urine voided/kg body weight was high compared with that from other horses and ponies. The mean urine specific gravity value was lower, and the mean urine pH higher, than published data.

The urinary excretion of sodium, potassium, chloride, inorganic phosphate, selected nitrogenous compounds and net acid/base were investigated. It was demonstrated that the composition of a single urine sample in no way indicated

the composition of the pooled volume voided over a 24 hour period.

Faeces were collected over 24 hour periods and the weight and water content were measured. It was discovered that the mean faecal water losses/24 hours were similar in volume to the mean urine volume voided/24 hours. Mean pH values of faecal fluids were acid in contrast to the marked alkalinity of urine. The quantities of sodium, potassium, chloride, ammonium, urea and net acid/base lost in faecal fluid were less than the respective quantities of these substances lost in urine. The inorganic phosphate lost in faecal fluid was much greater than that in urine, though the proportion of faecal fluid inorganic phosphate which represented that not absorbed was unknown.

The estimated intake of sodium, potassium and chloride by the ponies was compared with the output in urine and faecal fluid. It was found that of the sodium ingested 37% was excreted in urine and 16% in faecal fluid, leaving 47% unaccounted for. Potassium excretion amounted to 65% of that ingested, of which 52% was lost via urine, and 13% in faecal fluid. Of the estimated chloride ingested 78% was recovered, of which 75% appeared in urine, and 3% in faecal fluid.

The wide variations in the parameters investigated in the blood, urine and faecal fluids of these ponies illustrated the strong possibility of an erroneous judgement arising if an attempt to assess normality or abnormality was made on the basis of a single observation.

SECTION No.2

THE EFFECT OF FEEDING UPON THE PACKED CELL VOLUME PERCENTAGE AND THE CONCENTRATIONS IN PLASMA OF UREA AND SELECTED ELECTROLYTES.

INTRODUCTION

Ranges of the concentrations of selected parameters in blood collected from five Shetland and Shetland cross ponies have been described (see Section No.1 Part 1 and Appendix No.1). The results obtained showed wide ranges of values both between and within the untreated individual ponies. Thus, before proceeding further with experimental work it was considered necessary to study the effect of feeding upon the blood parameters which were to be investigated in later work in order that any changes observed were not erroneously attributed to experimental treatments. The blood samples analysed in the course of the work described in Section No.1 were collected at various times during the day, both before and after feeding, and it seemed possible that feeding could affect the packed cell volume percentage, and the concentrations in plasma of various electrolytes and urea. Urine collections were made on a 24 hour basis, so variations over shorter time period were immaterial.

No reference to the possibility of feeding causing rapid fluctuations in blood constituents was discovered, though some publications had a limited bearing upon the problem. Tasker[88] investigated the effect of thirsting and fasting, but his observations were made daily, rather than hourly, and his work did not include measuring the effects of administering food.

A rather more comprehensive study of changes in selected blood constituents in fasted ponies was made by Baetz and Pearson[107], and although blood samples were collected twice daily, this study, like Tasker's[88], involved starvation over several days, following which the ponies were observed when feeding recommenced. Baetz and Pearson[107] noted decreases in the concentrations of blood urea nitrogen and serum phosphorus during fasting, but no significant changes in serum chloride levels were observed. These workers postulated that the fall in blood urea nitrogen could be caused by renal loss or loss through saliva, and the decrease in serum phosphorus concentration was believed to be most likely due to renal loss and lack of intestinal absorption. Though the work of Baetz and Pearson[107] was relevant to the observations made upon the five Shetland and Shetland-cross ponies during the eight hours before feeding, their investigation and the experiment about to be described were not otherwise considered to be closely related. Ponies fed once every 24 hours will almost certainly still be digesting the previous feed(s) when the next daily ration is presented, whereas digestion in horses which have been deprived of food for nine days will have virtually ceased. It was anticipated, therefore, that any differences observed in a parameter before and after feeding would be smaller in magnitude than those noted by Baetz and Pearson[107].

Though it is not difficult to monitor changes in peripheral blood, the inaccessibility of most regions of the digestive tract renders monitoring concurrent changes

in the fluid and electrolyte contents therein impossible in the absence of fistulae, and it can be argued that a fistulated pony is not clinically normal. Alexander's[23] study of the electrolyte content of equine digestive tract fluids was confined to the analyses of specimens from newly slaughtered animals, and so the possibility of rapid post-mortem changes affecting the composition of the gut fluids cannot be completely disregarded. No reference to the examination of concurrent changes in the composition of blood and digestive tract fluids in equines was discovered. Alexander has measured the volume and electrolyte and urea content of salive produced by the parotid salivary gland over a twenty-four hour period[55]. Furthermore, he observed that saliva flowed only during mastication, and the faster the flow of saliva the higher were the concentrations of sodium, potassium, chloride and bicarbonate therein, though urea concentration was apparently insignificantly affected by flow rate.

Alexander and Benzie[108] showed that a barium meal passed through the stomach of a weaned foal very quickly, and reached the ileum within 30 minutes of ingestion, and in another publication Alexander[109] was able to demonstrate, using a fistulated pony maintained on a diet of hay, that the food could reach the large intestine within three hours of ingestion. This suggests that ingesta sometimes, but not invariably, traverses the small intestine quite rapidly. Unfortunately, concurrent changes in the electrolyte and urea content of peripheral plasma were beyond the scope of

his investigation.

It was discovered[102 192] that equine pancreatic secretion, unlike equine saliva secretion, may arise spontaneously, though vagus stimulation markedly increases pancreatic secretion. Alexander's[103 109] discoveries suggest that pancreatic secretion is likely to be maximal after a minimum of two hours beyond the commencement of the pony eating.

METHODS

The five ponies studied were those described in Section No.1. Their general management was also described in Section No.1.

Owing to the length of time the ponies were studied both before and after feeding, it was necessary to divide the work performed into two separate halves.

Throughout both halves of the experiments the ponies were housed in small looseboxes, the floors of which were devoid of covering in order that no bedding material could be eaten. The ponies had free access to drinking water at all times. The usual feeding time was 16.00 hours, so that for the first part of the experiment no readjustment period was necessary. Beginning at approximately 09.00 hours, hourly blood samples were collected by jugular venepuncture as described in Section No.1, Part 1. The ponies were offered food immediately after the eighth and last blood sample had been taken.

For the second part of the study it was considered necessary to allow the ponies to adjust to being fed at approximately 09.00 hours. Feeding time was therefore changed accordingly for five days before the day upon which the pony was studied. On the day of the study blood samples were collected hourly for eight hours, beginning one hour after the daily ration of hay was presented to the pony.

4 kg hay (the usual daily allowance) was carefully weighed and then offered to the pony. The hay net was

secured sufficiently far away from the source of drinking water to ensure that no hay fell therein. As soon as an hourly blood sample had been obtained the net containing the hay was weighed and the weight of hay consumed per hour was calculated. Any hay on the loosebox floor was replaced in the net before the latter was weighed. Thus, after eight hours the total weight of hay consumed was known, and the pattern of consumption was also established. The fistulated pony received the oats/bran supplement[55] after the last blood sample had been collected.

Packed cell volume percentages and the concentrations in plasma of sodium, potassium, chloride, inorganic phosphate and urea were determined in each blood sample collected, using methods already described in Part 1 of Section No.1.

RESULTS

Each of the five ponies was used as his own control. Individual results are tabulated in Appendix No.4. The quantities of hay consumed are also listed in Appendix No.4. The combined results ($\bar{x} \pm SD$) are presented in Table No.53.

The results of the analyses of blood samples collected after feeding were compared with those obtained from the blood samples collected before feeding. For each parameter measured in the individual pony the "after-feeding" results were divided into those obtained over the period extending from 1 to 4 hours after food was offered, and those obtained over the period extending from 5 to 8 hours after the presentation of food. For each set the mean $\pm$ SD was calculated, and compared with the mean $\pm$ SD of the "before feeding" results, using Student's "t" test, to determine whether the parameters measured differed significantly before and after feeding.

The statistical significance of the variations discovered is presented in Table No.54. Differences between ponies in the quantity of hay consumed were observed, though all ponies ate more hay/hour during the first half of the eight hour period than during the second half. No pony consumed all the hay offered.

Three ponies exhibited increased packed cell volume percentages after feeding commenced. The plasma urea concentrations of four ponies increased very markedly during feeding, though in one of the four the increase

TABLE No.53

THE EFFECT OF FEEDING UPON THE PACKED CELL VOLUME PERCENTAGE AND THE CONCENTRATIONS IN PLASMA OF UREA, SODIUM, POTASSIUM CHLORIDE AND INORGANIC PHOSPHATE. ($\bar{x}$ = S.D. n = 4)

Time		PCV %	Urea (mg/100 ml)	Na (mEq/l)	K (mEq/l)	Cl (mEq/l)	Inorganic P (mgP/100 ml)
Hours Before Feeding	8	32.0 ± 5.5	33.8 ± 19.9	133 ± 3	4.15 ± 0.40	101 ± 3	3.21 ± 0.82
	7	29.5 ± 4.0	34.5 ± 19.8	132 ± 2	4.00 ± 0.90	103 ± 4	3.25 ± 1.41
	6	31.0 ± 4.5	34.9 ± 20.7	131 ± 4	3.85 ± 0.35	102 ± 4	3.32 ± 0.98
	5	30.0 ± 4.5	35.4 ± 21.4	131 ± 3	3.85 ± 0.40	102 ± 4	3.35 ± 0.10
	4	30.0 ± 5.0	37.9 ± 21.4	131 ± 4	3.90 ± 0.15	102 ± 5	3.24 ± 0.22
	3	31.0 ± 6.5	37.9 ± 21.0	131 ± 4	3.90 ± 0.25	102 ± 4	3.43 ± 1.17
	2	31.5 ± 6.0	38.9 ± 21.8	132 ± 2	3.90 ± 0.50	103 ± 6	3.92 ± 0.80
	1	32.5 ± 6.5	37.4 ± 20.9	133 ± 3	3.80 ± 0.35	103 ± 5	3.44 ± 1.01
Feeding							
Hours After Feeding	1	36.0 ± 1.5	45.9 ± 11.5	137 ± 2	3.75 ± 0.35	102 ± 1	3.20 ± 0.76
	2	37.5 ± 1.0	47.9 ± 12.2	135 ± 1	4.20 ± 0.85	103 ± 1	2.74 ± 0.87
	3	38.0 ± 1.0	56.7 ± 21.2	136 ± 4	4.15 ± 0.60	101 ± 2	2.57 ± 0.67
	4	37.0 ± 2.0	54.7 ± 15.6	134 ± 1	4.40 ± 0.40	102 ± 1	2.33 ± 0.70
	5	36.5 ± 2.0	57.8 ± 28.1	134 ± 1	4.25 ± 0.25	102 ± 1	2.30 ± 0.78
	6	37.5 ± 2.0	55.9 ± 22.6	134 ± 4	4.00 ± 0.90	100 ± 3	2.17 ± 0.73
	7	37.0 ± 1.5	59.6 ± 29.8	136 ± 3	3.45 ± 0.65	100 ± 1	2.26 ± 0.74
	8	36.5 ± 2.5	58.6 ± 27.2	136 ± 2	4.00 ± 0.70	100 ± 2	2.58 ± 0.71

TABLE No.54

THE STATISTICAL SIGNIFICANCE OF THE RESULTS OBTAINED FROM THE MEASUREMENT OF THE PACKED CELL VOLUME PERCENTAGE, AND THE CONCENTRATIONS IN PLASMA OF UREA, SODIUM, POTASSIUM, CHLORIDE AND INORGANIC PHOSPHATE BEFORE AND DURING FEEDING

Parameter		Scruffy	Jimmie	Billie	Ben	MacGowan
Packed Cell Volume %	(1)	NS	↑ $p<0.05$	↑ $p<0.05$	NS	↑ $p<0.01$
	(2)	NS	↑ $p<0.05$	↑ $p<0.05$	NS	↑ $p<0.01$
Plasma Urea Concentration	(1)	↑ $p<0.01$	NS	↑ $p<0.01$	↑ $p<0.01$	NS
	(2)	↑ $p<0.01$	↑ $p<0.01$	↑ $p<0.01$	↑ $p<0.01$	NS
Plasma Sodium Concentration	(1)	NS	NS	↑ $p<0.05$	NS	NS
	(2)	NS	NS	↑ $p<0.05$	NS	NS
Plasma Potassium Concentration	(1)	NS	NS	NS	NS	NS
	(2)	NS	NS	NS	NS	NS
Plasma Chloride Concentration	(1)	NS	NS	↓ $p<0.05$	NS	NS
	(2)	NS	NS	↓ $p<0.05$	NS	NS
Plasma Inorganic Phosphate Concentration	(1)	NS	NS	↓ $p<0.05$	NS	NS
	(2)	↓ $p<0.05$	NS	↓ $p<0.05$	↓ $p<0.05$	NS

Key to the symbols used in Table No.54.

(1)=Results obtained from blood samples collected from 1 to 4 hours after food was presented.

(2)=Results obtained from blood samples collected from 5 to 8 hours after food was presented

NS = No statistically significant difference ($p>0.05$)

↑ $p<0.05$ Significant increase

↓ $p<0.05$ Significant decrease

↑ $p<0.01$ Highly significant increase

occurred only during the second half of the feeding period. The plasma sodium concentration of one pony increased during feeding and his plasma chloride concentration decreased concurrently. No changes in the plasma potassium concentration of any pony was noted. Decreases in plasma inorganic phosphate concentrations of three ponies occurred during feeding but in two of these three ponies the decrease was confined to the second half of the feeding period.

DISCUSSION

Though it was believed that the ponies had adapted to the change of feeding time by the end of the period of readjustment they were afforded, their behaviour throughout the eight hour feeding period was somewhat unexpected. They all showed a similar pattern of ingesting more hay in the earlier stages of the second half of the experiment than in the later stages. This was anticipated from previous observations, but their failure to consume the full 4 kg over the eight hours during which they were observed was surprising. It had been noted that the daily hay ration was usually totally consumed within 6 to 7 hours of feeding commencing, and only one pony (Scruffy) occasionally took longer.

Although too short a readjustment period might have been a contributory cause of the slow consumption of hay, it was believed that the major reasons were the distractions of regular blood sampling and temporary removal of the hay for weighing, and the almost constant presence of attendants. Normally the ponies were left unattended with their food.

Because many variable factors could not be eliminated careful interpretation of the results is necessary. It was impossible to force feed the hay to the ponies, and so variations in the quantities of hay consumed were unavoidable. The ponies' weights differed, and the hay consumed over the eight hour period ranged from 14.0 to 16.5 g/kg body weight. Since the ponies' plasma volumes were not identical (see

Section No.3) either in terms of absolute volume or ml/kg body weight, some lack of uniformity in the magnitude of plasma electrolyte and urea concentration changes would be expected for this reason too. Hence, each pony was used as his own control.

The amount of material from the previous day's food still being digested in the distal regions of the gastro-intestinal tract was unknown, and this most likely introduced another variable factor. The ponies were allowed free access to drinking water because it was considered that withholding water would introduced an artifact. The usual pattern of drinking was observed to be small volumes of water imbibed fairly frequently, and it appeared probable that the ponies simply maintained a fairly constant state of hydration.

An initial increase in the packed cell volume percentage at the commencement of feeding was expected, due to the excitement caused by the presentation of hay[60 61], but the increase was maintained throughout the duration of the time the ponies were observed. In two of the five ponies the increase was insignificantly small, in another two it was statistically significant ($p<0.05$) and in the fifth pony it was highly significant ($p<0.01$). However, changes in packed cell volume percentage may arise either because of changes in the cellular or non-cellular components of blood[8 12 18 60 61]. It seems likely that increases in the fluid content of the digestive tract would occur owing to the flow of digestive juices in response to

ingestion of food. Consequently a degree of haemoconcentration could result, as well as an increase in circulating erythrocytes caused by excitement. However, without directly measuring both plasma and erythrocyte volumes, the changes responsible for the increased packed cell volume percentages can only be surmised.

Christopherson and Webster[110], using T-1824 dye, were able to show that in sheep the plasma volume decreased sharply when feeding began, and slowly returned to the pre-feeding level after food was removed. The haematocrit value rose, and the authors believed this was due to an increase in the numbers of circulating erythrocytes, as well as a decrease in plasma volume. Christopherson and Webster[110], were unable to obtain accurate measurements of ECFV because thiocyanate entered the sheeps' saliva, and total body water was not measured, but they proposed that the decrease in plasma volume underestimated the flow of fluid into the gut. They suggested that the plasma deficits were minimised by restoration of the loss with intracellular water.

Christopherson and Webster quoted Bailey's[111], and Stacey and Warner's[112] discovery that a copious volume of fluid enters the digestive tract during digestion, but in the case of sheep, it was believed that the greatest influx was via the saliva, and the rumen wall[113].

All five ponies exhibited increases in plasma urea concentration after feeding, though in one pony the increases were too small to be statistically significant. The sudden onset of this increase is difficult to understand.

Even if, as postulated earlier, some degree of plasma concentration occurred when eating commenced and digestion continued, the magnitude of some of the increases strongly suggests that far more than a decrease in plasma volume was involved, since no other constituent measured showed such a pronounced change. Alexander and Davies[103] found urea present throughout the gastro-intestinal tract, and presumably some of this would be available for absorption, though it is probable that if absorption does occur, it would be a fairly continuous process. Houpt and Houpt[114] suggested that both endogenous and exogenous urea could be utilised by ponies fed on a low protein diet.

However, the rapidity with which increase in plasma urea concentration occurred after feeding commenced is not satisfactorily explained by any of these observations. It appears from the results obtained in the course of this investigation that urea formation in the liver from products of protein digestion and the subsequent circulation of the urea is the most likely major cause of the observed plasma urea levels. It has been demonstrated that the enzyme content of pancreatic fluid was low,[102 192] even in the pancreatic juice secreted during feeding, though no specific mention was made of whether this applied to proteolytic enzymes as well as amylase. Nevertheless, the discovery does not exclude the likelihood of enzymes from the ileum acting.

Though there was a trend towards a slight increase in plasma sodium concentrations after feeding, the increase

was statistically significant in only one pony. Because this increase was observed so quickly after eating commenced, it was unlikely to have been caused by the absorption of sodium from the hay. Two other possibilities exist. Sodium could be absorbed from the remains of earlier feeds, and peak absorption could have coincided with the ingestion of a subsequent feed. Alexander[23] demonstrated a sodium conservation mechanism in the equine gut. He[23] showed that the sodium concentration in the liquid of the small colon was lower than in saliva[55], pancreatic juice[102 192], and in the other regions of the gut he examined. Furthermore, it was shown in Section No.1 of this thesis that the mean sodium loss in faeces from these ponies was only 51 (± 35 S.D) mEq/24 hours. Thus it appears that sodium secreted into the more proximal regions of the gut is absorbed in the small colon. This would likely be a fairly continuous process, but maybe it would be increased if large quantities of ingesta were present in the distal regions of the gut. Tasker[86], also proposed that a strict sodium economy must act in horses, though he offered less evidence than Alexander[23].

The second possibility is that the decrease in plasma volume which has been postulated could cause a concurrent increase in the plasma sodium levels if sodium is not lost in isotonic quantities. A plasma water deficit is likely to be minimised by changes in other body fluid "compartments"[115], but a time lag might arise before the deficit is completely eliminated, especially if the digestive tract is still drawing large volumes of fluid.

Despite the high potassium content of hay[97] no statistically significant changes in plasma potassium concentrations occurred after feeding. However renal potassium excretion is also high (see Section No.1). This suggests that absorption of potassium is closely paralleled with renal excretion. Where slight increases were observed after feeding began, it is possible that either absorption of potassium (maybe from a previous feed) temporarily exceeded excretion, or water loss from plasma was responsible, or both.

Since serum bicarbonate concentrations were not monitored during this investigation the presence or absence of an alkaline tide was not established. No reference to this phenomenon in equines was discovered, though an increase in free plasma bicarbonate during eating has been reported in sheep[110].

Alexander[55], established that the mean concentration of chloride in saliva was approximately half that in plasma, which in view of the volume of saliva produced[55], represents a large quantity of this element. Furthermore, Comline et al.,[192] demonstrated a very high chloride concentration in equine pancreatic secretion. Nevertheless there were no distinct trends in plasma chloride changes before and after feeding. Only in one pony did statistically significant falls ($p<0.05$) occur after feeding.

Alexander[23] found a mean chloride concentration of only 6 mEq/l in fluid of the small colon. The mean total 24 hour faecal chloride loss from these ponies was also

low, i.e. 21 mEq (± 30 mEq S.D.) (See Section No.1). It was thus concluded that chloride conservation as well as sodium conservation occurs in equines, and secretion, re-absorption and renal excretion closely regulate the plasma concentrations of chloride.

In all the ponies the plasma inorganic phosphate levels were higher before feeding than afterwards. The observation was contrary to that of Baetz and Pearson[107], though the discrepancy may have arisen due to the longer period of starvation of Baetz and Pearson's animals. Otherwise this observation is difficult to explain. The inorganic phosphate content of plasma is low compared with sodium and chloride, but Alexander[23] discovered that inorganic phosphate concentrations were higher in the dorsal and small colon. Thus the maintenance of these high levels by the movement of inorganic phosphate into the lumen of the digestive tract might have made heavy demands upon the body resources which were reflected by decreases in the plasma levels.

It has been shown that a barium meal can reach the dorsal colon of a weaned foal within eight hours of administration[108]. However, the decreases in the plasma inorganic phosphate concentrations of these ponies began within 1 to 2 hours of feeding commencing. Furthermore, it is probable that not only could speed of the progression of digesta along the digestive tract of a weaned foal differ to that in the adult pony, but also that the consistency of the food ingested could affect the rate of movement too.

Though Alexander[109] showed that hay could reach the large intestine of a pony within three hours of ingestion digesta still has to traverse the proximal regions of the colon before reaching the dorsal colon. If phosphate secretion into the lumen of the colon is responsible for the decreases which were observed in plasma inorganic phosphate concentrations, and if this secretion is stimulated by the presence of digesta in this region of the gut, it may be that digesta from previous feeds caused the decreases observed. Alternatively, phosphate secretion into the more proximal regions of the gut may have caused the decreases in the concentrations of plasma inorganic phosphate which were observed so quickly after feeding commenced.

Because of the fluctuations observed in the blood constituents examined it was considered essential that in subsequent work considerable care should be exercised to standardise the times of blood sample collections in relation to feeding.

SUMMARY OF SECTION No.2

The influence of the short-term effect of feeding upon selected blood constituents was examined. All five ponies exhibited increased venous packed cell volume percentages during feeding. Four of the five ponies exhibited highly significant increases in plasma urea concentration after feeding began. Only one pony showed a significant increase in plasma sodium concentrations after feeding commenced, and this pony's plasma chloride level decreased concurrently. Plasma potassium concentrations were apparently unaffected. Three ponies showed decreased plasma inorganic phosphate levels during feeding.

It was concluded that standardisation of blood sampling time with respect to feeding time was essential when the effect upon the aforementioned blood constituents (other than potassium) of any experimental treatment was to be studied.

SECTION No.3

THE PLASMA AND "THIOCYANATE SPACE" VOLUMES OF THE UNTREATED PONIES. THE EFFECT OF THE ORAL ADMINISTRATION OF 7.5 LITRES OF WATER UPON THESE VOLUMES, AND UPON SELECTED BLOOD AND URINE CONSTITUENTS.

INTRODUCTION

References to the evolution and application of various methods used to measure body fluid volumes, and to their advantages and disadvantages, are too numerous to mention individually. Only those with a direct bearing upon the work performed with the ponies will be described and discussed. Most measurements of body fluid compartments have been carried out on small animals and man, but a few workers have applied some of the available methods to the measurement of body fluid volumes in equine animals, and special mention will be made of their work.

Modern methods of determining the volumes of the body fluid compartments are based on the dilution principle. By determining the dilution factor, the volume of diluent can thus be calculated. In their paper describing the estimation of the state of hydration of the body by measuring the amount of water available for the solution of sodium thiocyanate, Crandall and Anderson[116] listed the six major requirements of substances used to measure the volume of a body fluid compartment by the dilution technique. These requirements are:-

1) That it should be non-toxic in the dose and administration route involved.
2) That it should diffuse and equilibrate rapidly with water in the body fluid to be measured.
3) That its rate of equilibration should be rapid compared with its rate of excretion.

4) That the substance must not itself affect the state of hydration of the subject.

5) That the substance must not be adsorbed onto any other substances, nor must it be appreciably soluble in lipoids.

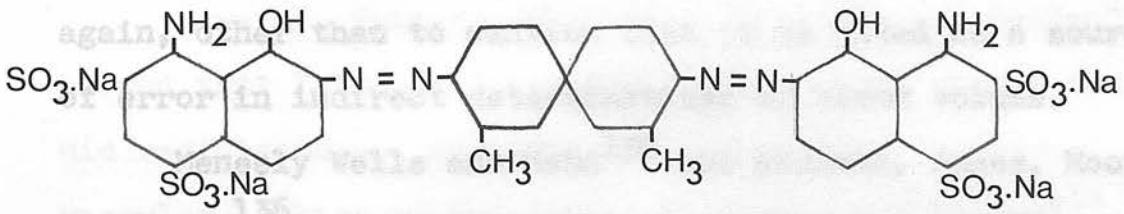
6) Accurate quantitative measurements of the substance must be possible.

Substances which have been used to measure plasma volume have included distilled water[117], pneumococcus polysaccharide[118], various colloids[119] [117], and proteins[120], haemoglobin[121] and carbon monoxide[122], but in more recent times radioactive tracers and dyes, or substances which, though not themselves dyes, will react to produce a quantitative colorimetric response, have been utilised. Although radioactive tracers now appear to be used more extensively than dyes and colour-producing reagents, this review will be confined largely to the second group of substances, since it was two such reagents which were used in the work performed upon the ponies.

(i) Plasma Volume Determination

Dawson, Evans and Whipple[123] studied the behaviour of a large number of dyes introduced into the circulation for the purpose of measuring plasma volume, and from their investigations they concluded that it was impossible to predict the behaviour of a dye in the bloodstream solely from a knowledge of its chemical formula. After testing over sixty dyes in dogs, they found that the dye - T-1824 was the most satisfactory of those they examined.

This dye, with the formula



is a water stable, water soluble azo dye with a molecular weight of 960[124]. First used for plasma volume measurements by Keith, Rowntree and Geraghty[125] it fulfilled the requirements listed by Crandall and Anderson[116] reasonably well. It had the added advantage that at the optimum light absorption of 620 nm the presence of a small degree of haemolysis did not significantly affect the optical density reading[18 123 126 127 128 129].

Despite the many advantages of using T-1824 for plasma volume determinations, it became evident that the dye was not ideal, and many workers encountered difficulties, and obtained wide variations in results when T-1824 was used[130 131 132 133 134]. It was later discovered, however, that not all the errors reported were intrinsically due to properties of the dye[20 135].

Large errors were incurred when attempts were made to determine blood volume by direct measurements of plasma volume and the packed cell volume percentage, and the subsequent calculation of red cell volume and blood volume

from the two direct observations[130]. The phenomenon of plasma trapping in the column of erythrocytes has already been discussed in Section No.1, and will not be discussed again, other than to mention that it is cited as a source of error in indirect determinations of blood volume.

Meneely Wells and Hahn[130] and Nachman, James, Moore and Evans[136], discovered that measuring plasma volume and packed cell volume and then calculating blood volume from such measurements overestimated the blood volume of human subjects. Suffice it to say that blood volume calculated from the direct measurement of either the cellular or the non-cellular fraction, and the venous packed cell volume percentage, may be considered a useful general indication of blood volume, especially if the value of the direct measurement is determined by collecting several samples and extrapolating the disappearance curve to zero. Where accuracy rather than speed is the primary aim, however, independent direct measurement of the cellular and non-cellular components appears to be a prerequisite[20 137 138].

A further source of error is the discrepancy between the venous haematocrit and the "total body" haematocrit. With one exception, workers who have investigated this discrepancy have demonstrated that the venous haematocrit was higher than the total body haematocrit[9 18 20]. Deavers et al.,[17] found that in their eleven ponies the converse was true, though the discrepancy was not large. This suggested that errors in trying to determine blood volume from measurements of plasma volume and venous packed cell

volume percentage were due to an inaccurate assessment of erythrocyte volume rather than lack of reliability of the dye dilution technique.

However, Krieger, Stoorasli, Friedell and Holden[133] showed that T-1824 indicated a higher plasma volume than did radioisotopes, and this they attributed to extravascular leakage of the dye. Gregerson and Rawson[138] also obtained higher values for plasma volumes with T-1824 than with non-dye substances, and Boyd[131], working with sheep, reported that the results of plasma volume determinations made with T-1824 were both higher and less reproducible than the results yielded by using radioisotopes. This suggests a failure of the dye to conform to the properties required of a substance used for body fluid volume measurements.

Payne, Ryley and Gartner[134] and Rawson[139] showed that when injected intravenously T-1824 became bound to plasma albumins, and therefore the volume of distribution of T-1824 in fact measured the volume of distribution of plasma albumin, though none of these workers proposed that plasma volume differed significantly from plasma albumin distribution volume in clinically normal subjects. Rawson[139] was able to demonstrate that some globulin binding could also occur, but this seemed unlikely to be a source of appreciable error.

Allen, Ochoa, Roth and Gregerson[140] examined the spectral absorption of equal concentrations of T-1824 in plasma from various species, and they observed significant

differences in the wavelength of maximal absorption between species. In equine plasma they discovered that maximal absorption occurred over the range 620 to 622.5 nm, though small variations within the equine species were observed. These differences between and within species suggested to Allen et al.[140] that more than one type of linkage occurred in the binding of T-1824 with plasma albumin.

Rawson[139] also demonstrated that the addition of plasma containing T-1824 to aqueous solutions alters the wavelength of maximal absorption. Furthermore, it has been shown[141] that the optical density of T-1824 can be affected by the salt concentration of solutions, pH values and protein concentrations though only in extremes incompatible with life. Thus it is important that the diluent employed for the final dilution of the dyed plasma prior to spectrophotometric determination of the optical density is selected with care. Payne et al.[134] concluded that the three major causes of error which occurred in plasma volume determinations were extravascation of the albumin plus dye, the turnover of plasma albumin and the consequent removal of the dye from the circulation, and removal of unbound dye by reticulo-endothial cells.

An alleged source of error which attracted much investigation was the so-called "Cat Effect" reported by Cruikshank and Whitfield[132]. Cruikshank and Whitfield showed that after intravenous injection of the dye into cats mixing was complete within approximately one minute, but they discovered that the dye disappearance

curves continued to fall rapidly for over six minutes, until a slower, steadier disappearance rate became evident. They then demonstrated that when a second injection was given the rapid component of the disappearance curve (i.e. the component which occurred after initial mixing and before the slow disappearance curve) was absent. They concluded that phagocytosis of some of the dye by reticulo-endothelial cells was responsible for the rapid disappearance of some of the dye after mixing. Cruikshank and Whitfield[132] found that unless the reticuloendothial system was "blocked" before a plasma volume determination with T-1824 was attempted the volume measured by extrapolation to zero time of the disappearance curve was subject to an error which could exceed twenty per cent. The phenomenon was denied in humans by Campbell, Sokalchuk and Penman[142] and by Noble and Gregerson[143] in humans and dogs. The possibility of this occurring in ponies has not apparently been investigated.

Conflicting opinions about whether several blood samples should be withdrawn after the injection of the dye, or whether a single sample is sufficient, are evident throughout the literature pertaining to plasma volume determinations. Obviously the single sample technique has the practical advantages that the plasma volume is determined relatively quickly, less work is involved, and the procedure is doubtlessly more comfortable and less alarming for the subject. However, the single sample technique appears to be more susceptible to error than the multiple sampling

technique. If a single sample is used, it is essential that this is not collected before mixing is complete[18 20 128 132 137 144] and ideally either no elimination of the dye should have occurred[128 144], or else an allowance should be made for it[20]. If several samples are collected the form of the disappearance curve should indicate if the first samples were collected too quickly following the injection of the dye, and appropriate action can subsequently be taken. Hahn, Ross, Bale, Balfour and Whipple[144] defined three phases of the T-1824 disappearance curve. The first phase corresponds with turbulent mixing in large vessels and axial streams of smaller vessels, the second phase the diffusion of the dye into sluggishly moving and stationary plasma films, and the third the loss of dye from the circulation. However, the calculation of plasma volume based upon the extrapolation of the clearance curve to zero time presumes that over the time of the investigation plasma volume does not change[117 124].

Several workers have applied dye dilution techniques to the measurement of equine plasma volumes[17 18 135 137 145] and others have used radioisotopes for the same purpose, and for the measurement of erythrocyte and blood volumes[7 9 17 20 146 147]. A summary of their results is presented in Table No.55.

Courtice[137] calculated what he believed to be the blood volume of his horses from the plasma volume and packed cell volume percentages. Although he based his calculations upon the results obtained from a single blood

TABLE No.55

EQUINE ERYTHROCYTE PLASMA AND BLOOD VOLUMES CITED IN PUBLISHED WORK

Reference	Erythrocyte volume (ml/kg body wt.)	Plasma volume (ml/kg body wt.)	Blood volume (ml/kg body wt.)	No. Observed	Type of Animal
7		53			Horses, Ponies, Donkey, Jennet
9	39.8	63.3	101.3	34	T.B. Racehorses
	25.3	52.2	77.5	6	Saddle Horses
	18.2	43.5	61.4	14	Percheron X.
17	20.7±2.94 S.D.	50.8±5.12 S.D.	71.5±6.28 S.D.	11	Small Ponies
18		51.7	105.7	21	20 Standard breds 1 T.B.
20	[a]29.1±7.0 S.D.		[a]82.6±12.5 S.D.		
	[b]30.4±7.2 S.D.		[b]82.7±11.2 S.D.		
135		52.37	81.14	1	T.B.
137	21	51	72	2	
145		49 ± 1.45 S.D.	73 ± 10.5 S.D.	12	Clydesdales and Clydesdale X
146			89*		
			101+		
147	47.1	61.9	109.6	6	"Hot Blooded" Horses
	28.5	43.2	71.7	4	Percherons

Key to Symbols used in Table No.55: a = Erythrocyte volume measured with ^{51}Cr-tagged erythrocytes.
b = Erythrocyte volume measured with ^{32}P-tagged erythrocytes.
* "Active" blood volume
+ "Total" blood volume.

sample, he stated that for work where accuracy rather than speed was the main objective, multiple sampling and extrapolation of the clearance curve to zero was preferable. Courtice[137] discovered that deeply pigmented equine plasma could affect the accuracy of the method. Using T-1824 Courtice[137] obtained variable day-to-day results from the same horse but he attributed these variations primarily to changes in blood volume.

Cronin[135] used a similar technique to that employed by Courtice[137] except that he collected several blood samples following the injection of the dye, and based his calculations on the extrapolation to zero time of the dye concentration curve. Cronin also applied a 4% "correction factor" for trapped plasma to the packed cell volume percentage value he obtained. T-1824 disappeared from the plasma of Cronin's horse at the rate of 4.37% of the injected dose/hour. Cronin[135] admitted that the assumption that 4% represented the true magnitude of the plasma trapped in the erythrocyte column of the haematocrit, and the use of a mean packed cell volume percentage for the calculation of the blood volume, were likely to be two sources of error in his work. Nevertheless he appeared unaware that venous haematocrit and body haematocrit were unlikely to be identical[9 17 18 20]. Furthermore, he accepted a basic presumption of the extrapolation method of calculating plasma volume - namely that the plasma volume remains unchanged throughout the period of time during which samples were withdrawn.

Collery and Keating[7], measured the erythrocyte volume of their animals using ^{32}P-tagged erythrocytes, and estimated blood and plasma volumes from the erythrocyte volume and the packed cell volume percentage. Though they "corrected" the observed packed cell volume percentage for trapped plasma they too assumed that venous and body haematocrit were identical[9 17 18 20].

Julian, Lawrence, Berlin and Hyde[147] also used ^{32}P-tagged erythrocytes to measure erythrocyte volume, but they failed to specify whether plasma volume was estimated or measured, though they did report a lack of reliability in the venous haematocrit as an indication of total blood and erythrocyte volume. They attributed this observation to the fact that changes in the cellular and non-cellular components of blood could occur independently and simultaneously.

Dalton and Fisher[145] used T-1824 to measure the plasma volumes of their horses. Their calculations appeared to be based upon the dye concentration in a single plasma sample. For calculating blood volume they assumed the body and venous haematocrit to be similar[9 18 20], and disregarded trapped plasma in the erythrocyte column[7 9 20 62 64 65 66 67].

One aspect of the work of Marcilese, Valsecchi, Figueiras, Camberos and Varela[9] has already been described in Section No.1. During the course of demonstrating discrepancies between total body haematocrit and venous haematocrit values, Marcilese et al.,[9] made direct simultaneous measurements of plasma and erythrocyte volumes

using ^{59}Fe and ^{51}Cr. Blood volume was calculated by summation of the plasma and erythrocyte volume. Their work demonstrated the higher erythrocyte and plasma volumes of thoroughbred horses compared with riding and draught type animals.

Zeller and Siewert[146] "labelled" serum albumin with ^{131}I and measured the volume of distribution of serum albumin - ^{131}I in blood. They investigated the horses both in the resting state and after exercise, and they termed the blood volume they measured in the animals at rest the active blood volume. The volume after exercise was termed the total blood volume and was the larger of the two volumes. Zeller and Siewert[146] considered that the total blood volume represented the volume of circulating blood in the resting animal plus the volume contained in "blood reservoirs" which they believed emptied as a result of strenuous exercise.

In the course of determining erythrocyte, plasma and blood volumes of domestic animals, including horses, Lauder[20] encountered many sources of error associated with the dilution technique. He discovered that when the cellular and non-cellular components of blood were measured directly the results differed from those obtained by indirect estimation. Lauder[20] also discovered that different tracers yielded different results, but he expressed the belief that the most consistent sources of error in the dilution technique was the failure to estimate precisely the quantity of material injected. Mention was also made of the

error involved in calculating blood and blood constituent volumes from the concentration of injected material in a single blood sample as opposed to several. Lauder[20] found that T-1824, though reasonably satisfactory, could be affected by haemolysis and lipaemia, whereas ^{131}I had the advantage of being affected by neither, though it suffered other disadvantages.

Persson[18] considered that T-1824 met the usual requirements of a substance used for dilution techniques[116] and in addition, unlike radioisotopes, it had the advantage of almost unlimited storage. Persson[18] stressed that the difference between body haemotocrit and venous haematocrit was large in horses, and this he attributed to the spleen acting as a reservoir for blood with a very high haematocrit. Persson[18] used a 1% solution of T-1824 to determine the plasma volumes of his horses, and based his calculations upon the concentration of the dye in the plasma of a blood sample collected 15 minutes after the injection of the dye. He listed the following sources of error in his work as being:-

a) perivascular infiltration,
b) incorrect volume injected,
c) haemolysis, though he considered this relatively unimportant,
d) attempting to derive blood volume from the plasma volume measured with T-1824, and the venous packed cell volume percentage.

Persson[18] calculated that the percentage error in

plasma volume determinations in the horse, using T-1824 was likely to be approximately ± 8.4%, and if blood volume was then estimated from plasma volume and the packed cell volume percentage, the likely error would be circa 12.5%. Persson[18] noted that excitement, nervousness and exercise elicited changes in the dye concentration in plasma, and he presumed that these reflected changes in plasma volume, which thus appeared to decrease under such circumstances. Increases in the packed cell volume percentage, reaching a maximum after 4 minutes, were concurrent with these emotions, and Persson[18] attributed this principally to splenic contraction. Persson[18] claimed that the blood volume of the horse was more closely correlated with the animal's body weight than with its body surface area, though it is possible that difficulty in obtaining an accurate assessment of the surface area of the body of a horse could be a complicating factor.

Bianca's[156] investigation of the effects of dehydration, rehydration and overhydration were confined to cattle, but as will be evident later in this section, he observed similar phenomena to those observed when the ponies were water-loaded, so mention will be made of Bianca's work. As he anticipated, dehydration caused an increase in the packed cell volume percentage, total blood solids, haemoglobin concentration, blood viscosity and erythrocyte total solids. Overdehydration, effected by the infusion into the rumen of 5% to 10% body weight of water, caused initial increases in packed cell volume percentage, total blood solids and

haemoglobin concentration, which later fell to normal. During overhydration, stress caused by the infusion was postulated as the cause of the observed increases. Since Bianca[156] failed to observe any indication of excitement he believed that stress caused the mobilisation of erythrocyte-rich blood into the circulation, and, simultaneously, water from plasma into tissue spaces.

Bianca[156] also noted changes in the urine produced by the ruminants during variations in their states of hydration. Dehydration was observed to cause an increase in urine specific gravity. Overhydration caused a fall in the specific gravity of the urine voided within approximately 1½ hours of the water loading. This lower urine specific gravity was maintained for approximately 4 hours before the return to normal began, and within the three hours following the infusion a volume of urine equivalent to 18% to 43% of the water infused had been passed.

(ii) "Thiocyanate Space" Volume Determination

No substance has been found to possess the properties which make it ideal for use in the measurement of extracellular fluid volume by the dilution technique[148]. Furthermore, the definition of extracellular fluid is inconsistent[128 149]. Hix, Evans and Underjberg[149] defined extracallular water as the water contained in plasma, lymph, and interstitial fluid, whilst others[128] included cerebrospinal fluids to which Hix et al.[149] referred as transcellular water. Doubtlessly it was convenient for most

workers to include in their definition of extracellular fluid those body fluid compartments entered by substance they were using during their investigations.

Sodium thiocyanate was the substance chosen by many. Although not ideal, it fulfilled Crandall and Anderson's[116] criteria for substances used to measure a body fluid volume by the dilution technique. To avoid confusion with true extracellular fluid volume, the volume of fluid through which sodium thiocyanate diffuses will be referred to as the "thiocyanate space".

Sodium thiocyanate is non-toxic in the dose range normally used, reasonably rapid in reaching equilibrium, slow to be excreted, and not normally metabolised. Crandall and Anderson[116] believed that sodium thiocyanate did not significantly affect the state of hydration of the subject. They also believed that it was not bound to any serum fraction precipitated by trichloracetic acid, though conflicting evidence is available from other authors[151 152].

Following the intravenous injection of sodium thiocyanate Crandall and Anderson[116] discovered its presence in lymph, cerebro-spinal fluid and salivary and pancreatic secretions, though the concentrations of thiocyanate in these fluids were not identical with that in plasma. Unfortunately Crandall and Anderson[116] did not state the species to which these discoveries applied. Although Crandall and Anderson[116] found that the fluid volume through which thiocyanate was distributed in their horses was equivalent to 27.5% of the animals' body weight, no detailed

reference to the equine body fluids into which thiocyanate entered was discovered. It appeared that all work of this nature has been confined to other species.

Gregerson and Stewart[153] discovered that sodium thiocyanate and T-1824 could be simultaneously used for measuring so-called "thiocyanate space" and plasma volume respectively, since trichloracetic acid precipitated the T-1824 along with plasma proteins, leaving the thiocyanate ion in the supernatant fluid. Another advantage of the use of thiocyanate in this way was that serum containing the ion could be stored unchanged for up to one month[153]. Though such a long storage period was unnecessary in the course of the work with the Shetland ponies, the fact that thiocyanate and T-1824 solutions could be used simultaneously was a great advantage.

Although Winkler, Elkington and Eisenman[154] doubted that the dilution volume of the thiocyanate ion co-incided exactly with the extracellular fluid volume, they nevertheless believed it to be a useful approximation because they considered that changes in the volume of the "thiocyanate space" may reflect changes in true extracellular fluid volume. The relationship between the "thiocyanate space" and extracellular fluid volume is unknown. Elkington and Taffel[155] believed that the volume of distribution of thiocyanate increased when several hours have elapsed following thiocyanate injection, due to the entry of thiocyanate ions into cells. Nevertheless Elkington and Taffel[155] advocated multiple sampling, provided the samples are collected before

the time after which thiocyanate is believed to enter the cells, rather than the single sample technique. The work of Rosenbaum and Lavietes[150] showed that the thiocyanate ion entered erythrocytes proportionately to the water content of the erythrocytes. It was also found to enter some cells, notably those of glandular organs. Though this work was not performed upon horses, it seems possible that it could apply to equine species. Rosenbaum and Lavietes[150] supplied strong evidence of the formation of a lipoid-thiocyanate complex after intravenous injection of sodium thiocyanate, so that a portion of thiocyanate was not free to diffuse from plasma.

Scatchard, Scheinberg and Armstrong[151] investigated the combination of thiocyanate ions with serum albumin, and discovered that as many as 40 thiocyanate ions could combine with each albumin molecule, though they emphasised that species differences could arise. Scheinberg and Kowalski[152] examined thiocyanate binding further, and discovered that in human serum approximately one half of the total thiocyanate administered was bound, mostly to serum albumin, but also to another serum component and/or erythrocytes. If binding occurs in equine serum it is possible that a different proportion of the thiocyanate might be bound, but, until it can be shown that this does not occur in horses, it is reasonable to regard thiocyanate binding as a source of error in the measurement of the thiocyanate space. Scheiberg and Kowalski[152] stated that the relationship between the so-called "thiocyanate space" and true extracellular fluid

volume is unknown, for the following reasons:-

a) the concentration of extracellular albumin is unknown.

b) the extent to which thiocyanate enters cells is unknown.

c) equilibrium between the thiocyanate in plasma and that in extracellular fluid may not be attained.

Regarding a) Scheinberg and Kowalski[152] concluded that the closer the concentration of albumin in extracellular fluid approached that in plasma, the smaller the error due to differences in albumin concentrations would be. Regarding b) because thiocyanate ions enter the erythrocytes in concentrations similar to the free thiocyanate concentration in serum, and because thiocyanate ions enter other cells too, a falsely high result is incurred. Regarding c), since plasma or serum are the only extracellular fluids normally available for sampling, the concentration of thiocyanate in interstitial fluid is unknown, and if the concentration in plasma or serum remains higher than in interstitial fluid then a falsely low value for the volume of extracellular fluid is obtained.

Little work appears to have been undertaken which involved investigating the magnitude of the extracellular fluid volume or the thiocyanate space in equines. The work of Crandall and Anderson[116] has already been mentioned. Yousef, Dill and Mayes[115] studied shifts in the body fluids of donkeys during dehydration. They discovered that the percentage reduction in the plasma volume was less than that in the thiocyanate space volume, which in turn was less than the percentage decrease in the intracellular fluid

volume. Yousef et al.[115] concluded that the pattern of change was effective in minimising reductions in circulating blood volume at the expense of thiocyanate space fluid and intracellular fluid. No evidence of overhydration was present after access to water was permitted.

It has been recorded that the rapid ingestion of one litre of water in man induced an 8% increase in the thiocyanate space without a detectable change in the plasma volume arising[159]. No reference to the measurement of the body "fluid compartments" of equines subjected to water loading has been discovered, however.

The measurement of the plasma and "thiocyanate space" volume of the untreated ponies was undertaken in order to determine not only the magnitude of the volumes, but also the percentage of the body weight which plasma comprised. In addition, it was of interest to compare the plasma volumes of these ponies with those published by others who investigated the body "fluid compartments" of equines.

The determination of the plasma volumes made possible the estimation of the approximate total quantities of certain electrolytes and urea in plasma. Hence, on future occasions, when changes were noted in the concentrations of these plasma constituents, it was possible to relate changes in concentration to probable changes in the total quantities present. Obviously it was only possible to obtain approximate values because considerable variations in electrolyte

and urea concentrations have been shown to arise spontaneously (see Section No.1, Part 1), and after feeding (see Section No.2).

A study of changes in the body "fluid compartments" following the administration of a large volume of water per os was considered to be a useful exercise because of possible clinical applications. It was believed that the expansion of the plasma volume in this manner might, if the expansion proved to be sufficiently large, be a simple and rapid way of treating dehydration in an animal unable to drink voluntarily. Furthermore it was considered possible that changes in the concentrations of selected blood constituents after water loading might reflect the percentage changes in the plasma volume as indicated by T-1824 dilution. It was also of interest to examine the effect of the rapid intake of a large volume of water upon the volume, specific gravity, pH and selected constituents of the urine subsequently voided.

METHODS

The five ponies studied during this series of experiments were those which were used throughout the work described in this thesis. Basic management has been described previously.

Each pony was subjected to two investigations, both of which involved the measurement of the plasma volume with T-1824 dye, and the measurement of the "thiocyanage space" volume with sodium thiocyanate. In all the experiments 10 ml of a 1% or 2% solution of T-1824 dye, and 40 mls of a 10% sodium thiocyanate solution were injected intravenously into a jugular vein. The T-1824 was dissolved in physiological saline solution, and the sodium thiocyanate in sterile distilled water. It was found necessary to increase the concentration of the T-1824 solution from 1% to 2% because of fluctuations in the optical density of undyed plasma. This increase satisfactorily minimised the effect of the fluctuations. All blood samples were collected by venepuncture of the opposite jugular vein to that into which the T-1824 dye and sodium thiocyanate were injected, using B-D vacutainers containing freeze-dried heparin anticoagulant, and a B-D 20G needle.

During the first series of experiments the plasma and thiocyanate space volumes in the untreated ponies were measured. Throughout the duration of the experiments free access to drinking water was permitted. Hay was offered after the final blood sample was collected.

A blood sample was withdrawn, and approximately 1.5 ml of a 2% xylocaine solution was infiltrated into the skin

over a jugular vein. Five minutes later a 16G needle was inserted into the vein, and a free flow of blood through the needle was considered to indicate complete penetration into the vein. A syringe containing the T-1824 dye was attached to the needle, and the dye was injected as rapidly as possible. A brief pause followed, during which the syringe, with the plunger fully depressed, was held onto the needle in order to minimise the loss of dye-rich blood. The syringe was then removed and replaced with another containing the sodium thiocyanate solution. The injection procedure was repeated. Commencing one hour after the injection of T-1824 and sodium thiocyanate eight blood samples were collected at hourly intervals.

Immediately after the collection of the blood samples the packed cell volume percentages were measured in triplicate in the manner described in Section No.1, Part 1, of this thesis. The remaining blood was then centrifuged at 4,000 r.p.m. for 15 minutes, and the plasma was decanted off the cells into a tube which was stoppered and stored at $4^{o}C$. The optical density of T-1824 in the plasma samples was determined by the method described by Visscher[128].

Visscher's[128] method for the quantitative determination of thiocyanate in plasma was modified slightly for this work. In order to attain higher concentrations of thiocyanate in the final solutions, 2 ml of plasma was added to 8 ml of trichloroacetic acid. 2 ml of the standard solution was likewise added to 8 ml of trichloroacetic acid. The solutions which were subjected to optical density

measurement comprised 4 ml of supernatant fluid and 3 ml of ferric nitrate reagent. This small modification ensured that the deflection on the optical density gauge of the spectrophotometer always remained within the 0.300 to 0.800 range, where sensitivity was greatest. The optical density was read at 622.5 nm. All optical density measurements were made using a Unicam SP500 spectrophotometer.

The concentrations of T-1824 dye and thiocyanate in plasma were calculated from the optical density measurements. The results were plotted graphically. The $\log_{10}$ of concentrations of T-1824 or thiocyanate were plotted on the ordinate, and a linear time scale on the abscissa. The lines of best fit were calculated using the method of least squares, and the lines were extrapolated to the y axis of the graph, in order that the "zero-time" concentration could be determined.

Plasma and thiocyanate space volumes were calculated using the equation

$$v = \frac{x}{y} \text{ litres}$$

where v = the plasma or "thiocyanate space" volume (litres)

x = the quantity of T-1824 or thiocyanate injected (mg)

y = the plasma concentration of T-1824 or thiocyanate at "zero-time" as indicated by the intersection of the line of best fit with the y axis of the graph (mg/litre).

The second series of experiments involved the measurement of changes in plasma and thiocyanate space volumes following the rapid administration of 7.5 litres of water

by stomach tube. This volume is equivalent to about 4% body weight, and approximately 1.5 x the plasma volume of the individual ponies. Changes in the packed cell volume percentages and the concentrations of selected plasma constituents were also studied. Urine was collected for analysis for 48 hours after water loading took place. The collection of urine necessitated the use of the urine collecting apparatus[94] and the ponies stood in stalls for the duration of the collection. Access to drinking water was permitted, and the daily hay ration was presented after the collection of the penultimate blood sample.

In order to establish the plasma and thiocyanate space volumes immediately before treatment six blood samples were collected before the water was administered. Of these samples, one was removed before the injection of T-1824 and sodium thiocyanate, to serve as a source of undyed plasma, and five were obtained afterwards. Immediately after sample 6 was taken, a stomach tube was passed and 7.5 litres of water were administered as rapidly as it would flow down the tube. This procedure took 5 to 8 minutes. As soon as the stomach tube was removed the delivery tube of the urine collecting apparatus[94] was connected to the collecting bottle.

A summary of the times of the blood sampling and the analyses performed upon the individual blood samples is presented in Table No.56.

Packed cell volume percentages and the concentrations of selected electrolytes were determined as described in

TABLE No.56.

EXPERIMENTAL PROTOCOL - WATER LOADING EXPERIMENTS

Sample No.	Time of Blood Sampling	Measurements made on Sample
1	Before T-1824 and NaSCN injection	PCV, Plasma Constituents, Blanks for T-1824 and SCN^-
2	20 minutes after injection	PCV, T-1824, SCN^-
3	1 hour after injection	PCV, T-1824, SCN^-
4	1½ hours after injection	PCV, T-1824, SCN^-
5	1¾ hours after injection	PCV, T-1824, SCN^-
6	2 hours after injection	PCV, T-1824, SCN^-
7	2¼ hours after injection	PCV, T-1824, SCN^-
8	2½ hours after injection	PCV, Plasma Constituents, T-1824, SCN^-
9	2¾ hours after injection	PCV, T-1824, SCN^-
10	3 hours after injection	PCV, Plasma Constituents, T-1824, SCN^-
11	3½ hours after injection	PCV, T-1824, SCN^-
12	4 hours after injection	PCV, Plasma Constituents, T-1824, SCN^-
13	6 hours after injection	PCV, Plasma Constituents, T-1824, SCN^-
14	7½ hours after injection	PCV, T-1824, SCN^-
15	8 hours after injection	PCV, Plasma Constituents, T-1824, SCN^-
16	24 hours after injection	PCV, Plasma Constituents.

Section No.1 of this thesis. The determination of the optical density of T-1824 and ferric thiocyanate and the calculation of their concentrations in plasma has already been described. Before calculating the change in the volumes of plasma and "thiocyanate space" the graph was examined visually. Unless the first five points formed a reasonably straight line, attempts to determine the degree of change in the volume of plasma and thiocyanate space were abandoned. If the graph appeared satisfactory, calculations were continued. The graphs of both T-1824 and SCN^- concentrations in plasma showed a similar general form, illustrated in the diagram. The derivation of the equation for calculating increases in the body fluid compartments is shown.

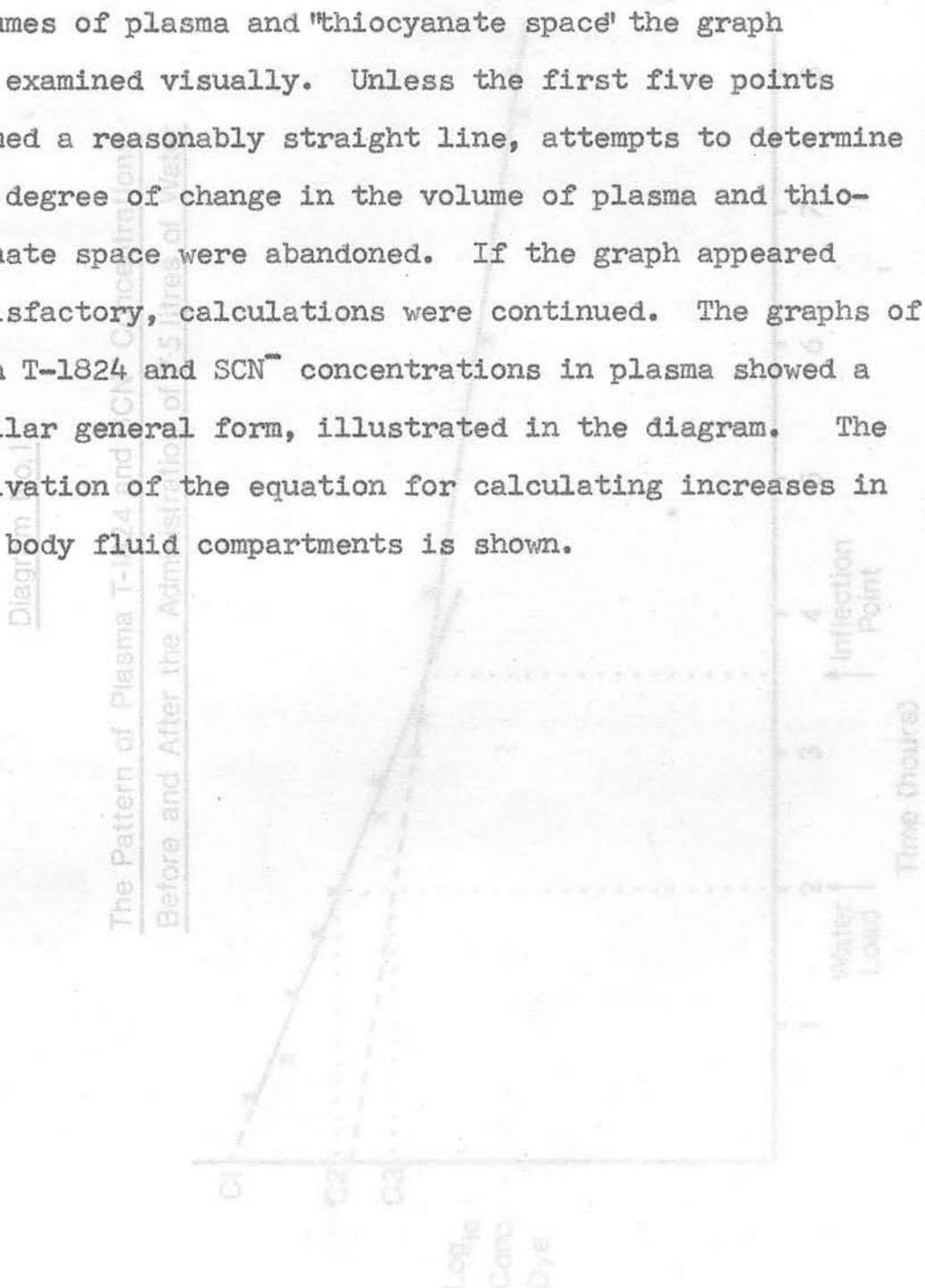
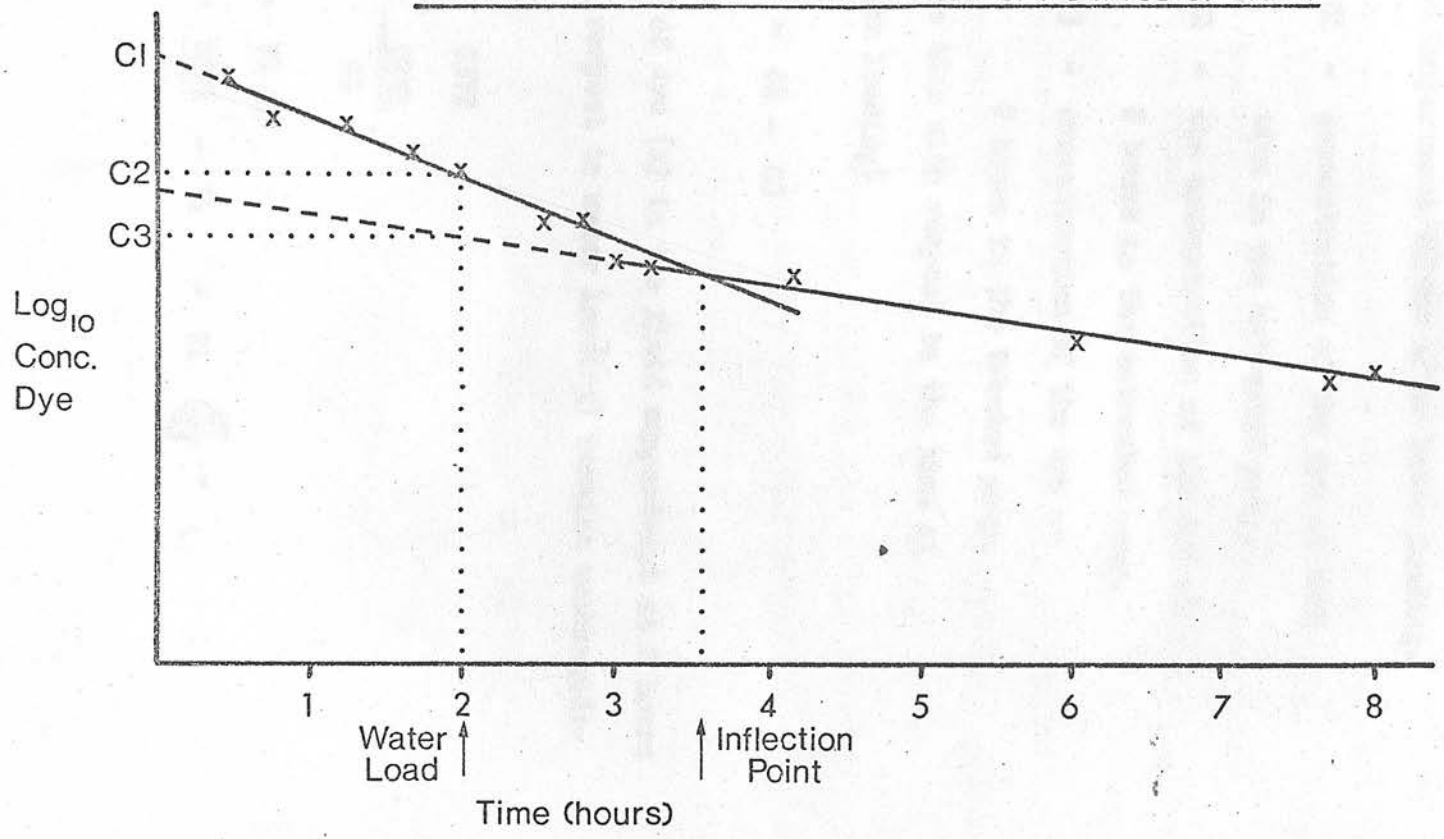


Diagram No. I

The Pattern of Plasma T-1824 and SCN^- Concentrations
Before and After the Administration of 7·5 litres of Water



Explanation

The Calculation of the Expansion in the Volume of a Body Fluid Compartment

Let x = the quantity of dye present at 2 hours.

Let V1 = the Fluid Compartment Volume before Water Loading.

Let V2 = the Fluid Compartment Volume after Water Loading.

By extrapolation, C1 = concentration of the dye at zero time in the untreated pony.

By extrapolation, C2 = the concentration of the dye at 2 hours in the untreated pony.

By extrapolation, C3 = concentration of the dye at 2 hours in the treated pony.

(N.B. 2 hours = zero time with respect to the time of water loading)

$$\Delta c = C2 - C3$$

However the quantity of dye (x) in the fluid compartment at 2 hours (i.e. zero time with respect to water loading) remains unchanged:-

$$\therefore C2V1 = C3V2$$

$$\therefore V2 = \frac{C2V1}{C3}$$

Increase in volume = V2-V1

$$= \frac{C2V1}{C3} - V1 = V1\left(\frac{C2}{C3} - 1\right)$$

Two lines of best fit were derived using the method of least squares. As can be seen in the diagram, two components of the concentration curve were present, but since the demarkation between the two was seldom distinct it was necessary to add and subtract points in order to determine to which component of the curve those concentrations in the post water-loading period belonged. The changes in the volumes of plasma and thiocyanate space were calculated as shown in the explanation following the diagram.

Urine analyses were performed using the methods described in Section No.1, Part 2, of this thesis. Owing to the urine collecting bottles having a capacity of 9 litres it was necessary to collect the urine passed during the first 24 hours after the administration of the water load in two aliquots.

RESULTS

The data collected from the study of the plasma and thiocyanate space volumes of the untreated ponies are presented in Tables 57 and 58. Presented in Tables 59 and 60 are the data obtained from the investigation of the effect of the rapid administration of 7.5 litres of water upon these volumes. The approximate total quantities in plasma of selected electrolytes and urea are listed in Table No.61. The effect of the water load upon selected blood constituents is presented in Tables 62 to 67. The effects of the water load upon 24 hour urine volume, pH, specific gravity and selected constituents are presented in Tables 68 to 76. In order that differences between individual ponies were not masked, each pony was used as his own control throughout all this work.

After water loading discomfort was manifest by sweating in four out of the five ponies. Of the four, one pony (Jimmie), on whom sweating was especially pronounced, exhibited paddling movements of the limbs, suggestive of mild colic. The ponies were noticed to sometimes turn and bite their flanks, and all five ponies defaecated within three minutes of receiving the water load. Signs of discomfort subsided within approximately 2 hours of water loading. The daily hay ration was offered six hours after water loading, and all the hay was consumed within 17 hours.

N.B. The plasma and thiocyanate space volumes measured in the untreated ponies over an eight hour period will be referred to as "control" volumes. The results obtained by

extrapolation of the clearance curves to zero for the two hours immediately preceding water loading will be referred to as the "before water loading" volumes. Those results obtained immediately following the administration of 7.5 litres of water will be referred to as the expanded volumes.

TABLE No.57

THE PLASMA VOLUMES OF THE UNTREATED PONIES

	Scruffy	Jimmie	Billie	Ben	MacGowan
Plasma Vol.(l)	5.59+	5.20*	4.58+	4.97*	5.43*
Wt. of Pony (kg)	178.7	164.2	181.4	181.4	214.1
Plasma Vol. (ml/kg body wt.)	31.3	31.7	25.3	27.4	25.4
Specific gravity of plasma	1.026	1.026	1.027	1.024	1.027
Wt. of plasma(kg)	5.74	5.33	4.70	5.09	5.58
% Body Weight	3.21	3.25	2.60	2.81	2.61

\+ 1% T-1824 solution used

* 2% T-1824 solution used.

The plasma volumes showed no obvious relationship with body weight. The smallest pony (Jimmie) had the largest plasma volume/kg body weight, and the largest pony (MacGowan) the second smallest plasma volume/kg body weight. Though the range of the weights of the other three ponies was within 3 kg, differences in plasma volume of approximately 20% were observed.

The plasma of the smallest pony represented the

greatest percentage of the body weight. The second smallest pony had the second largest percentage body weight of plasma. In the remaining three ponies no relationship between the body weight and the percentage of the body weight represented by plasma was evident.

TABLE No.58

THE "THIOCYANATE SPACE" VOLUMES OF THE UNTREATED PONIES

	Scruffy	Jimmie	Billie	Ben	MacGowan
SCN^- Space Vol. (l)	42.59	32.25	41.90	48.64	45.94
Wt. of pony (kg)	178.7	164.2	181.4	181.4	214.1
SCN^- Space Vol. (ml/kg body wt.)	238.3	196.4	231.0	268.1	214.6

Though the smallest pony had the smallest thiocyanate volume, and the smallest thiocyanate space volume/kg body weight, no relationship between the thiocyanate space volumes and the body weight of the other ponies emerged. The pony with the smallest thiocyanate space volume/kg body weight had the largest plasma volume/kg body weight.

TABLE No.59

THE EXPANSION OF THE PLASMA VOLUME BY A 7.5 l WATER LOAD

	Scruffy	Jimmie	Billie	Ben	MacGowan
*Plasma vol. before H_2O load (l)	6.16	5.78	5.70	5.42	6.36
Plasma vol. before H_2O load (ml/kg body wt.)	34.47	35.20	31.42	29.88	29.71
Expanded Plasma vol (l)	6.60	6.27	6.02	5.50	6.45
Expanded plasma vol (ml/kg body wt.)	36.93	38.16	33.19	30.32	30.13
Weight of pony(kg)	178.7	164.2	181.4	181.4	214.1
Expansion (l)	0.44	0.49	0.32	0.08	0.09
Expansion (ml/kg body wt.)	2.46	2.98	1.76	0.44	0.42
% increase	7.1	8.5	5.6	1.5	1.4
Time after water loading of the inflection of the clearance curve	2h8min	1h17min	3h20min	1h27min	30min

*The volume measured in the 2 hours preceding the administration of the water load.

N.B. 2% T-1824 solution was used for these measurements.

The expanded plasma volumes of these ponies ranged from 0.08 l to 0.49 l greater than the volumes before water loading. Percentage increases varied from 1.4% to 8.5% which were equivalent to 1.1% to 6.5% of the water load administered. Because only one control and one experiment were performed upon each pony, the reliability and repeatability of the experimental method could not be checked.

In all ponies the difference in plasma volume before and after water loading was less than the differences observed between the control volume and the volume before expansion.

The greater the weight of the pony, the smaller was the increase in plasma volume/kg body weight, and the smaller the percentage increase in plasma volume. Though Billie and Ben fitted into this general pattern they exhibited different increases in plasma volume/kg body weight, and in the percentage increases in plasma volume, despite their body weights being identical.

In Scruffy and Jimmie close agreement in the "control" and the "before water loading" thiocyanate space volumes was obtained. In Billie and Ben the "before water loading" volumes were lower than the "control" volumes. In MacGowan the "before water loading" thiocyanate space volume was much greater than the "control" volume.

In Billie, Ben and MacGowan the magnitude of the expansion of the thiocyanate space volume was less than the difference in volume between the "control" and the "before water loading" volumes.

Except in the case of MacGowan, the expansion of the thiocyanate space volume, as indicated by inflection of the clearance curve, commenced before the expansion of the plasma volume. The largest pony (MacGowan) exhibited the greatest expansion of the thiocyanate space volume, the greatest increase/kg body weight and the greatest percentage increase. Otherwise there was no obvious relationship between the ponies' weights and the magnitude of the expansion of their

TABLE No.60

THE EXPANSION OF THE THIOCYANAGE SPACE VOLUME BY A 7.5 l WATER LOAD

	Scruffy	Jimmie	Billie	Ben	MacGowan
*SCN^- Space before the load (l)	42.19	29.07	30.82	35.49	62.61
SCN^- Space before the load (ml/kg body wt.)	236.09	177.04	169.90	195.64	292.43
Expanded SCN^- space (l)	47.95	32.41	34.96	37.97	76.14
Expanded SCN^- space (ml/kg body wt.)	268.32	197.38	192.72	209.30	355.62
Weight of pony (kg)	178.7	164.2	181.4	181.4	214.1
Expansion (l)	5.76	3.34	4.14	2.48	13.53
Expansion (ml/kg body wt.)	32.2	20.3	22.8	13.7	63.2
% Increase	13.7	11.5	13.4	7.0	21.6
Time after water loading of inflection of the clearance curve	1hr33min	54min	51min	1h12min	1h21min

* The volume measured in the two hours preceding the administration of the water load.

thiocyanate space volumes. In the largest pony the expansion of the thiocyanate space volume exceeded the volume of the water load.

In all ponies the percentage increase in the thiocyanate space volume was greater than the percentage increase in the plasma volume. The ratio of the increase in plasma volume:increase in thiocyanate space volume ranged from 1:6.8 to 1:148.3. The former ratio was observed in the smallest pony, and the latter ratio in the largest pony. The ratio of the percentage change in plasma volume: percentage change in thiocyanate volume ranged from 1:1.4 to 1:15.4. The largest ratio was observed in the smallest pony, and the smallest in the largest pony.

A trend towards these ratios decreasing as the body weight increased was evident, though no direct relationship was apparent.

TABLE No.61

THE APPROXIMATE TOTAL QUANTITIES IN PLASMA OF SELECTED ELECTROLYTES AND UREA

	Scruffy	Jimmie	Billie	Ben	MacGowan
Vol. of Plasma (l)	5.59	5.20	4.58	4.97	5.43
Sodium (mEq)	760	697	609	646	733
Potassium (mEq)	23	25	18	19	21
Chloride (mEq)	565	520	458	492	543
Bicarbonate (mEq)	160	145	127	134	153
Inorganic Phosphate (mgP)	173	165	142	177	134
Urea (mg)	1627	2948	1608	1551	1489

These results were derived from the mean values of the plasma constituents in the individual ponies (see Section No.1, Part 1) and the "control" plasma volumes. Because wide ranges in the concentrations of these constituents were observed in the untreated ponies it was possible to calculate only the approximate total quantities present in plasma. Changes in plasma volume may also cause fluctuations.

TABLE No.62

CHANGES IN PACKED CELL VOLUME PER CENT AFTER A 7.5 l WATER LOAD

Time	Scruffy	Jimmie	Billie	Ben	MacGowan
Before H_2O	34.5	33.0	31.0	32.0	33.0
½ hour after H_2O	31.5	32.0	31.0	36.0	36.0
1 hour after H_2O	31.0	32.5	30.0	33.0	36.0
2 hours after H_2O	33.5	32.5	29.0	29.0	33.5
4 hours after H_2O	34.0	32.5	30.0	33.0	35.5
6 hours after H_2O	32.0	36.5	32.0	46.0	32.5
24 hours after H_2O	33.5	34.5	27.0	30.5	33.5

No obvious consistent pattern of change in the packed cell volume percentage was observed. Only in Ben did the lowest packed cell volume percentage occur after the commencement of plasma volume expansion. (The low packed cell volume percentage observed in Billie 24 hours after water loading was disregarded.) Nearly all the values were

within the normal range of packed cell volume percentages of the untreated ponies.

TABLE No.63

CHANGES IN PLASMA SODIUM CONCENTRATION AFTER A 7.5 l WATER LOAD (mEq/l)

Time	Scruffy	Jimmie	Billie	Ben	MacGowan
Before H_2O	128	130	130	125	136
½ hour after H_2O	125	133	125	125	135
1 hour after H_2O	123	126	131	125	135
2 hours after H_2O	123	115	134	128	130
4 hours after H_2O	129	126	135	140	130
6 hours after H_2O	133	135	135	134	132
24 hours after H_2O	128	128	138	130	137

No consistent pattern of change in plasma sodium concentrations after water loading occurred. Ben's plasma sodium ooncentration showed no decline whatever. Scruffy and Billie showed small falls which occurred before the expansion of the plasma volumes began. Of the remaining two, Jimmie showed a pronounced decrease and MacGowan a smaller decrease, at varying times after water loading.

TABLE No.64

CHANGES IN PLASMA POTASSIUM CONCENTRATION AFTER A 7.5 l WATER LOAD (mEq/l)

Time	Scruffy	Jimmie	Billie	Ben	MacGowan
Before H_2O	4.60	4.10	4.50	4.00	4.10
½ hour after H_2O	4.50	4.10	3.90	3.70	4.10
1 hour after H_2O	4.10	4.00	3.90	3.50	3.70
2 hours after H_2O	4.20	4.10	3.30	3.35	3.85
4 hours after H_2O	3.80	4.15	3.90	3.60	3.95
6 hours after H_2O	3.80	4.00	3.00	3.45	4.00
24 hours after H_2O	4.30	4.10	4.00	4.20	3.60

Decreases in the concentration of plasma potassium were noted in all five ponies after water loading, but in four of the ponies the decreases commenced before the expansion of plasma volume was detectable.

TABLE No.65

CHANGES IN PLASMA CHLORIDE CONCENTRATION AFTER A 7.5 l WATER LOAD (mEq/l)

Time	Scruffy	Jimmie	Billie	Ben	MacGowan
Before H_2O	94	104	99	98	98
½ hour after H_2O	95	104	94	98	96
1 hour after H_2O	96	101	95	96	94
2 hours after H_2O	90	98	93	99	93
4 hours after H_2O	97	100	100	102	96
6 hours after H_2O	104	106	100	103	99
24 hours after H_2O	103	110	102	105	98

Though all ponies exhibited lower plasma chloride concentrations at some stage after water loading than beforehand, only in MacGowan did the onset of the decline in

the plasma chloride concentration coincide with the commencement of plasma volume expansion. The lowest concentration in Scruffy, Billie and Ben was attained before plasma volume expansion began. Jimmie's lowest plasma chloride concentration occurred after the expansion of his plasma volume began.

TABLE No.66

CHANGES IN PLASMA INORGANIC PHOSPHATE CONCENTRATION AFTER A 7.5 l WATER LOAD (mgP/100ml)

Time	Scruffy	Jimmie	Billie	Ben	MacGowan
Before H_2O	4.05	3.26	3.13	3.74	2.36
½ hour after H_2O	3.60	2.65	2.86	3.54	1.97
1 hour after H_2O	2.50	2.87	2.21	3.15	2.32
2 hours after H_2O	2.76	2.94	2.27	3.22	1.43
4 hours after H_2O	3.17	4.32	2.72	3.91	1.35
6 hours after H_2O	2.70	3.64	2.57	3.59	1.68
24 hours after H_2O	2.81	3.41	3.07	3.03	1.50

Decreases in the concentration in plasma of inorganic phosphate occurred in all ponies, but, with the exception of MacGowan, the decreases commenced and attained their lowest levels before plasma volume expansion began. The lowest concentration of plasma inorganic phosphate in MacGowan was not reached until 4 hours after water loading, i.e. 3½ hours after plasma volume expansion commenced.

TABLE No.67

CHANGES IN PLASMA UREA CONCENTRATION AFTER A 7.5 l WATER LOAD (mg urea/100 ml)

Time	Scruffy	Jimmie	Billie	Ben	MacGowan
Before H_2O	29.9	51.4	31.9	31.5	25.2
½ hour after H_2O	28.2	45.6	35.4	35.7	25.9
1 hour after H_2O	27.8	43.0	31.3	35.0	26.1
2 hours after H_2O	25.4	41.6	34.9	37.1	24.9
4 hours after H_2O	25.7	37.7	25.2	38.5	23.5
6 hours after H_2O	28.2	30.4	29.0	40.6	25.4
24 hours after H_2O	28.5	29.4	22.3	56.0	39.4

Pronounced differences between ponies in the plasma urea concentrations were evident. MacGowan exhibited no marked change in plasma urea concentration until 24 hours after the water load, when an increase arose. Ben exhibited a small rise in the plasma urea level ½ hour after being water loaded. Further small rises were evident from 4 hours to 6 hours, and then a large increase occurred 24 hours after the water load was administered. The plasma urea concentration of Scruffy declined progressively from immediately prior to water loading to 2 hours afterwards. The level remained low until 4 hours after water loading, and then began to increase slightly. Billie exhibited a substantially decreased plasma urea concentration 4 hours after water loading, and following a small rise at 6 hours, another decrease occurred which led to the lowest value observed in this pony, 24 hours after he received the water load. Jimmie's plasma urea concentration declined progressively over the whole 24 hours during which he was studied.

KEY TO THE SYMBOLS USED IN TABLES 68 TO 76.

* Denotes a significant increase ($p < 0.05$) above the mean value in the untreated pony.

\+ Denotes a highly significant increase ($p < 0.01$) above the mean value in the untreated pony.

/ Denotes a significant decrease ($p < 0.05$) below the mean value in the untreated pony.

x Denotes a highly significant decrease ($p < 0.01$) below the mean value in the untreated pony.

TABLE No.68

THE VOLUME OF URINE PASSED AFTER A 7.5 l WATER LOAD (ml)

Time after water load	Scruffy	Jimmie	Billie	Ben	MacGowan
0-10½h.	5890	4760	7260	9310	10020
10½h-24h	3830	1160	1440	860	1800
Total 0-24h.	9720*	5920*	8700+	10170+	11820+
24h-48h	5520	2040	3040	1270	1840

The volumes of urine voided by the ponies during the 24 hours following the administration of the water load were significantly increased above normal in two of the ponies (Scruffy and Jimmie) ($p < 0.05$) and highly significantly increased in the other three ($p < 0.01$). Of the urine voided during this time by far the greatest part was voided during the first 10½ hours. The urine volume passed by Jimmie was less than the volume of the water load administered.

The volumes of urine voided during the 24 to 48 hours following water loading were within normal limits, but in

all cases they were lower than the normal mean daily urine volumes passed by these ponies.

TABLE No.69

THE SPECIFIC GRAVITY OF URINE PASSED AFTER A 7.5 l WATER LOAD

Time after water load	Scruffy	Jimmie	Billie	Ben	MacGowan
0-10½h.	1.009	1.008[x]	1.006[+]	1.005[+]	1.005[+]
10½h-24h	1.013	1.026	1.030	1.041	1.033
24h-48h	1.015	1.035	1.022	1.048	1.045

Concurrent with the increased volumes of urine voided within the first 10½ hours of the collecting period were decreases in the specific gravity of the samples. The decreases were statistically significant in three ponies, highly significant in another, and insignificant in the fifth pony. The specific gravity of all urine samples collected during the latter half of the first day, and during the second day after water loading, fell within normal limits.

TABLE No.70

THE pH OF URINE PASSED AFTER A 7.5 l WATER LOAD

Time after water load	Scruffy	Jimmie	Billie	Ben	MacGowan
0-10½h.	8.40	8.80	9.15	8.80	9.00
10½h-24h	8.60	7.80	8.45	8.30	8.20
24-48h	8.80	8.30	9.00	8.80	8.35

No significant pH changes were noted in any urine sample collected during the two days following water loading.

TABLE No.71

THE URINARY EXCRETION OF SODIUM AFTER A 7.5 l WATER LOAD (mEq)

Time after water load	Scruffy	Jimmie	Billie	Ben	MacGowan
0-10½h.	29	157	120	410	331
10½-24h	25	41	58	26	29
0-24h	54	198	178	436+	340
24-48h	50	61	155	51	28

Four of the five ponies showed varying degrees of increased urinary sodium excretion during the 24 hours after the ingestion of the water load. In three of the four, the increases were not statistically significant, but in the fourth pony (Ben) the increase was highly significant ($p < 0.01$). During the second day following water loading the sodium excretion fell to within normal ranges, though the urinary sodium lost by all the ponies excepting Billie was below the normal mean daily loss.

TABLE No.72

THE URINARY EXCRETION OF POTASSIUM AFTER A 7.5 l WATER LOAD (mEq)

Time after water load	Scruffy	Jimmie	Billie	Ben	MacGowan
0-10½h.	589	309	363	233	551
10½-24h	575	302	432	292	549
0-24h	1164	611	795	525	1100
24-48h	911	836	517	533	801

The urinary excretion of potassium was not significantly affected by water loading, though a trend towards a low output was observed in three ponies. In Billie and Ben this trend continued into the second day. The low potassium content of the urine voided by Ben during the first day was accompanied by a significant increase in urinary sodium.

TABLE No.73

THE URINARY EXCRETION OF CHLORIDE AFTER A 7.5 l WATER LOAD (mEq)

Time after water load	Scruffy	Jimmie	Billie	Ben	MacGowan
0-10½h.	359	267	305	503	110
10½-24h	326	175	196	213	312
0-24h	685	442	501	716	422
24-48h	447	386	344	342	357

The urinary chloride excretion of these ponies was not significantly affected by water loading, though all ponies excreted less urinary chloride during the second day than during the first.

TABLE No.74

THE URINARY EXCRETION OF INORGANIC PHOSPHATE AFTER A 7.5 l WATER LOAD (mgP)

Time after water load	Scruffy	Jimmie	Billie	Ben	MacGowan
0-10½h	252.8	1077.7	63.6	196.1	10.3
10½-24h	182.3	240.3	30.8	259.7	24.5
0-24h	435.1+	1318.0+	94.4	455.8	34.8
24-48h	49.4	486.2*	55.0	263.2	21.1

In three ponies urinary inorganic phosphate excretion was not significantly changed after water loading, but Jimmie and Scruffy exhibited highly significant increases ($p < 0.01$) during the first 24 hours following water loading. Jimmie also excreted significantly more urinary inorganic phosphate ($p < 0.05$) during the second day following water loading.

TABLE No.75

THE URINARY EXCRETION OF AMMONIUM AFTER A 7.5 l WATER LOAD (mEq)

Time after water load	Scruffy	Jimmie	Billie	Ben	MacGowan
0–10½h	98	77	278	95	175
10½–24h	120	35	73	107	73
0–24h	218	112	351	202	248
24–48h	260	91	376	248	106

No significant deviations from the normal daily urinary ammonium excretion were observed in any pony during the 48 hours following the ingestion of the water load.

TABLE No.76

THE URINARY EXCRETION OF UREA AFTER A 7.5 l WATER LOAD (g)

Time after water load	Scruffy	Jimmie	Billie	Ben	MacGowan
0–10½h	20.8	10.4	14.1	14.6	12.8
10½–24h	18.0	6.1	18.3	11.5	21.8
0–24h	38.8	16.5	32.4	26.1	34.6
24–48h	28.6	11.9	24.9	22.6	29.0

In no pony was the daily urinary urea excretion significantly changed during the 48 hours following the water load.

DISCUSSION

Differences in the body weights of the ponies might be expected to influence the plasma volume, but no close correlation between plasma volume and body weight was apparent in these ponies. The differences between ponies in the plasma volume/kg body weight suggested that other factors influenced the plasma volumes of these ponies. The proportion of body fat has been shown to influence blood and plasma volumes[147], and variations in the body fat content of the ponies may have been partly responsible for the differences observed. Dehydration was excluded as a possible factor, though variations in the state of hydration could have arisen. Compared with the plasma volumes of equines studied by other workers (see Table No.55) the plasma volumes of these ponies were outstandingly low, although they bore closer resemblance to the plasma volumes of draught horses than light riding horses. It was anticipated that the plasma volumes of the ponies might be similar to those of the Shetland-type animals investigated by Deavers et al.[17], but Deavers' ponies also had markedly higher plasma volumes than the ponies studied in the course of this work. Without further investigation the reasons for these very low results can only be postulated, but it is suggested that the high proportion of body fat of the ponies[147], and a general lack of physical fitness compared with regularly exercised working animals[18], might have been contributory causes.

It is difficult to explain why the "control" plasma

volumes were consistently lower than the "before water loading" plasma volumes. All graphs of the T-1824 clearance curve yielded satisfactory straight lines. Furthermore, had two hours been inadequate for complete mixing of T-1824, such that the concentration in the fast-flowing regions of the circulation was greater than that in the slower-moving areas, lower results for the volumes preceding water loading would have been obtained[144]. The possibility that these results reflect natural variations cannot be eliminated, but this is difficult to accept in view of the consistent nature of the discrepancies, which also tends to exclude two other sources of error from being constantly involved - namely inaccurate assessment of the quantity of T-1824 injected[20] and perivascular infiltration[18 134]. In addition, the extrapolation technique, rather than single sampling was adopted throughout all this work, and of the two methods, the extrapolation technique should afford the greater degree of accuracy[18 137 138]. The "Cat Effect", if it did occur, was not considered to be a contributory cause of the discrepancies[132] since it would have affected both the control volumes and the volumes before water loading. Though several weeks elapsed between the determination of the "control" plasma volumes and the "before" and "after" water loading plasma volumes, body weight changes were negligible, so the discrepancies observed were not attributed to body weight increases.

It was concluded that a knowledge of the normal variations of the plasma volumes of the untreated ponies

is essential before further studies of factors disturbing plasma volume are investigated. This necessitates the measurement of the plasma volume to be repeated several times for each pony.

The unusually large volumes of urine voided by the ponies after water loading were considered to indicate that much of the water load had been absorbed, but this could not be accepted as proof that the plasma volumes had been expanded beyond their normal upper limits. Absorption could have occurred relatively slowly, and a tendency to a consequent increase in the plasma volume could have been counteracted by the passage of some of the water into other fluid compartments[16 115] and the renal excretion of excess water absorbed[156]. The inflections in the clearance curves were regarded as the primary evidence for increases in the plasma and thiocyanate space volumes after water loading.

It was probable that variations in the state of hydration of the ponies at the time of water loading influenced the extent of the expansion of the plasma and thiocyanate space volumes. The fact that one of the ponies (MacGowan) exhibited an increase in the thiocyanate space volume greater than the volume of the water load administered might have been due to part of a large volume of water already present in the intestine being absorbed along with a portion of the water load itself. Access to drinking water at all times was permitted because it was intended that the effects of water loading should be studied in

normal ponies. Withholding water beforehand would thus introduce a degree of abnormality.

Because of a lack of published data it is not known how closely the thiocyanate space volumes of these ponies compared with those of other equines. Since the specific gravity of the thiocyanate space fluid is unknown the thiocyanate space volume was not expressed as a percentage of the body weight[116].

Though discrepancies in the "control" and "before water loading" thiocyanate space volumes arose in four out of the five ponies the results obtained from Jimmie were considered to be within acceptable limits. The paired results from Billie, Ben and MacGowan all showed very large differences, the reasons for which are unknown, and these observations are difficult to understand. Since plasma volume and thiocyanate space volume were both determined by the dilution technique the same basic principles apply, and hence both determinations are susceptible to similar sources of error associated with dilution. These sources of error have already been described and discussed.

The determination of the normal variation in the thiocyanate space volume of the individual ponies would be necessary before the discrepancies observed in Billie, Ben and MacGowan could be established as normal or abnormal. It is probable that the differences observed may have been caused both by normal variation and by error, but it is believed that no single error was wholely responsible for the discrepancies.

It was possible that the large "before water loading" thiocyanate space volume of MacGowan (c.f. the control volume) was due to an unusually large volume of water in the intestine, into which the thiocyanage diffused[128]. A possible source of error which could have given rise to the lower "before water loading" thiocyanate space volume in Billie and Ben (cf. their control volumes) is incomplete diffusion of sodium thiocyanate during the two hours between the injection of the thiocyanate[116 128 157 158], though the clearance curves did not suggest this.

A very large error in the estimation of the volume of thiocyanate injected would have had to have been made for such a discrepancy in results to have been caused by the inaccurate assessment of the quantity of thiocyanate injected[20], and this was discounted. Since it was possible to visually detect as little as 0.5 ml to 1.0 ml of fluid infiltrated subcutaneously into the tissue of the jugular groove, the risk of not detecting a volume sufficient to cause such a large discrepancy was negligible.

From the observation that the percentage increases in the thiocyanate space volumes were greater than the increases in the plasma volumes it was concluded that the water absorbed was so distributed as to minimise changes in the plasma volume. Assuming that the erythrocyte volume remained unchanged, disturbanced in blood volume would therefore be minimal[115]. This observation is similar to that of Bellisario, Campa, Pugiliese, Giuliano, Giuffrida and Condorelli[159].

The inflection points om the T-1824 and thiocyanate clearance curves indicated that in four of the ponies the expansion of the thiocyanate space volume commenced before that of the plasma volume. It was concluded that initially, water absorbed into the bloodstream diffused into the thiocyanate space fluid (and probably the intracellular fluid also), and only after some expansion of the thiocyanate space volume was the plasma volume increased, either by retention of some of the absorbed water in the bloodstream or by back diffusion from the thiocyanate space.

From the investigations performed it was impossible to determine the duration of the expansion of the plasma and thiocyanate space volumes. The times of the first micturition after water loading varied from one to five hours. Though micturition may have indicated that the excretion of the water load had commenced, it is likely that the volume of urine within the bladder before water loading would have influenced these times.

Although the use of isotopes concurrently with T-1824 and thiocyanate would be valuable for some comparative purposes, radioisotopes used to measure extracellular fluid volume possess a different volume of dilution to thiocyanate[20 128 154 160 161]. The value of such an exercise would therefore be limited to investigating whether isotope dilution reflected the same magnitude of change as that indicated by dyes and related substances, and whether the dye and the isotope both indicated an identical time for the commencement of volume expansion after water loading.

Although the cellular and non-cellular fractions of the blood form two different morphological and physiological entities[8 12 18 60 61] the venous packed cell volume percentages were measured before and after water loading in order to determine whether any decreases occurred which reflected the increases in plasma volume indicated by the increased dilution of T-1824.

Since it has been shown that fear, excitement[60 61], and the time lapse between feeding and blood sampling can influence the packed cell volume percentage these had to be considered when interpreting the results. It was also appreciated that the venous packed cell volume percentage differed from the "total body haematocrit"[9 17 18 20] and hence changes detected in jugular packed cell volume percentages might not typify the overall change. A redistribution of erythrocytes from elsewhere in the circulation and/or an increase in total circulating erythrocytes brought about by splenic contraction[9 18 20 61] might have masked decreases in the venous packed cell volume percentage. Splenic contraction might have been mediated by apprehension and discomfort, the signs of which were evident after the water load had been administered[60 61 156].

A tendency to a decrease in the packed cell volume percentage could also have been masked by some increase in the red cell water content causing enlargement of the erythrocytes themselves[13]. Furthermore, the times at which the venous packed cell volume percentages were lowest frequently occurred before the commencement of plasma volume

expansion. The large increase in the venous packed cell volume percentage of Ben six hours after water loading was partially attributed to excitement in anticipation of feeding[60 61] since hay nets for other ponies were inadvertently produced while a blood sample was being collected.

It was concluded that despite a trend towards decreased packed cell volume percentages at various times during the six hours following water loading, changes in the venous packed cell volume percentages were not a true indication of concurrent changes in plasma volume. Furthermore, increases of 1.4 to 8.5% in the plasma volumes would be unlikely to induce changes in the packed cell volume percentage outwith the normal range. This observation accords with that of Bianca[156] who investigated some aspects of the effect of overhydrating cattle. It was also believed that changes in the venous packed cell volume percentage were not a true indication of the magnitude of change of the total volume of circulating erythrocytes, and an accurate assessment of such changes would necessitate direct measurement of the erythrocyte volume.

The pattern of change of some plasma constituents was complicated by factors other than a probable dilution of the plasma. The wide range of normal values has already been discussed in Section No.1 of this thesis, and the effect of feeding upon these values was demonstrated in Section No.2. Despite four of the five ponies exhibiting decreases in plasma sodium concentrations during the six

hours after receiving the water load, in no pony did the maximum percentage decrease correspond with the calculated percentage increase in the plasma volume. It was envisaged that sodium excretion could influence plasma sodium levels, and it was also considered possible that any tendency to a decline in plasma sodium concentration could be counteracted by the mobilisation of sodium from elsewhere in the body. Furthermore, the injection of sodium in the form of sodium thiocyanate could have maintained the plasma concentrations at the levels observed.

It appeared possible that dilution of the plasma could have exerted an effect upon the plasma potassium concentrations but if so, no distinct pattern emerged. The decreases observed in plasma potassium and chloride concentrations failed to correlate well with either the onset of plasma volume expansion or the degree of expansion.

No distinct trends in changes in the plasma inorganic phosphate concentrations of the ponies were detectable. Fluctuations appeared somewhat randomly, and decreases did not coincide with the onset of plasma volume expansion defined by the clearance curves of T-1824.

Because the sustained fall in the plasma urea concentration of Jimmie commenced within 30 minutes of his being water loaded, i.e. more quickly than the apparent expansion of the plasma volume, it was concluded that dilution of the plasma by the absorption of a portion of the water load was likely to be only partially responsible for the decrease in the plasma urea level. Furthermore, assuming that the

inflection point on the T-1824 clearance curve accurately indicated the commencement of plasma volume expansion, then volume expansion would only exert a detectable effect from two hours after water loading onwards.

From this investigation into the effect of water loading upon selected plasma constituents it was evident that no consistent well defined indications of plasma dilution were obtained by monitoring the levels of the blood constituents studied. As has been stated previously such factors as normal fluctuations, urinary excretion, the diffusion of some constituents into the plasma from cells, and the influence of the time lag between feeding and blood sampling, could well all affect the concentrations in plasma of the substances investigated. Percentage changes in the various plasma constituents of the individual ponies studied showed no close similarities.

Decreases in the constituents frequently commenced, and sometimes even reached their lowest levels, before the plasma volume expansion was detectable on the T-1824 clearance curves. Though the clearance curves may be susceptible to some errors[134], in view of the total lack of uniformity in the fluctuations of the plasma constituents monitored, the clearance curves appeared the more reliable of the two criteria. Thus it was concluded that the only valid and satisfactory way of measuring the plasma volume, and changes therein in these ponies, was by dye or isotope dilution. It is impossible to deduce from these data the quantity of water absorbed following the rapid administration

of 7.5 litres. A study of changes in total body water after water loading remains to be investigated at a future date.

It was not considered feasible to estimate the volume of water absorbed from the volume of urine voided for four reasons. Firstly, the volumes of urine passed/24 hours by the untreated ponies varied considerably within individuals, and so it was impossible to deduce the proportion of the urine voided which was derived from the water load. Secondly, the volume of urine passed over 24 hours only measured the rate of voidance, not the rate of urine production. The use of urinary catheters would have been necessary if urine production had been investigated. Thirdly, the renal route of excretion is not the only route of water loss, though it was the only route investigated. It was shown in Section No.1 Part 3 that faecal water loss/24 hours closely approximates to urinary water loss over the same period of time, and any attempt to balance water intake and output would need to include this loss. Loss of water due to the sensible sweating observed in four of the five ponies would also need to be measured. Fourthly, it was not proved that the whole of the water load absorbed was eliminated within 24 hours, despite the fact that the volumes of urine voided 24 to 48 hours after water loading were within normal limits.

Though 7.5 litres probably represents only a small percentage of the gut content[88 93] the fact that the intake was so rapid was believed to be the primary cause of the

subsequent diuresis. Because of the evidence for plasma volume expansion it was considered that diuresis was mediated by increases in glomerular filtration rate[162] and the suppression of antidiuretic hormone secretion[163 164 165]. Unfortunately, no specific references to the physiology of diuresis in the horse were discovered, and most of the work of this nature was performed upon man and small animals. The diuresis observed in these ponies bore a close resemblance to that observed by Bianca[156] following overhydration in cattle. The decreases in the specific gravity of the urine voided in the 10½ hours immediately after water loading accord with the observation that high rates of urine voidance are commonly associated with low specific gravity values[89].

The influence of increases in body water upon the composition of urine does not appear to have been investigated in the horse, and such work appeared to have been confined to man and laboratory animals. In these species it has been shown that aldosterone secretion is reduced by body fluid expansion[165]. Decreased proximal tubular absorption of sodium was observed in dogs following increases in extracellular fluid volume of approximately 3%[166], and reduced renal tubular sodium reabsorption in man was also noted following volume expansion[162]. Increases in renal plasma flow have been shown to decrease net tubular sodium reabsorption[167] and increases in glomerular filtration rate were believed to be involved too[162]. Some or all of these sodium regulatory mechanisms may have been operative in the ponies. It was also possible that some

of the sodium administered in the form of sodium thiocyanate contributed to the sodium content of the urine.

The reasons for the somewhat low urinary potassium excretion by three ponies are unknown. With the exception of one pony (MacGowan) a tendency towards an inverse relationship between renal sodium and renal potassium excretion was evident in the first 24 hours after water loading. This suggests that during diuresis sodium and potassium might compete for excretion.

A relationship between the tubular reabsorption of sodium and inorganic phosphate has been demonstrated in dogs[168]. Neither of the ponies whose urinary inorganic phosphate excretion increased after water loading showed a concurrent natriuresis. Furthermore, the pony who responded to water loading with a significant natriuresis failed to show an increase in urinary inorganic phosphate content. Thus the relationship described by Fulop and Brazeau[168] did not appear to extend to the ponies under these experimental conditions.

The lack of significant changes in urinary ammonium and urea excretion indicated that water loading had no effect upon these urine constituents.

It was observed that the faeces voided after water loading appeared to possess a higher water content, and it is reasonable to suspect that the quantities of some constituents would also have altered outwith the normal values. Such changes might therefore have been found to compensate for any changes observed in urine constituents.

Time did not permit a study of the effect of the rapid administration of 7.5 litres of water upon the water content, pH, and the quantities of selected constituents of faecal fluids, which remain to be investigated on a future occasion.

It was concluded that other than increasing the volume and decreasing the specific gravity of the urine subsequently voided, the effects upon the urine of these ponies of a 7.5 litre water load were few, and not sustained.

SUMMARY OF SECTION No.3

Plasma and "thiocyanate space" volumes were measured in the untreated ponies, with T-1824 dye and sodium thiocyanate respectively. The approximate total quantities of selected constituents in plasma were calculated from the plasma volume and the mean concentrations of the plasma constituents. The plasma and thiocyanate space volumes were also measured immediately before and after the rapid administration of 7.5 litres of water by stomach tube. Plasma and thiocyanate space volumes were increased after the ponies had been water loaded. The percentage increases in the plasma volumes ranged from 1.4% to 8.59%, and in the thiocyanate space volumes from 7.0% to 21.6%.

The venous packed cell volume percentages and the concentrations of selected plasma constituents did not accurately reflect the degree of plasma volume expansion indicated by T-1824. Furthermore, the fluctuations showed little or no similarity between ponies.

The volumes of urine produced during the 24 hours following water loading were significantly increased, and concurrent decreases in specific gravity were observed. After water loading there was a trend towards increased urinary sodium excretion in four of the five ponies, and in one pony the increase was highly significant. A trend towards a reduced urinary potassium output was observed, but this was statistically insignificant. Urinary inorganic phosphate excretion was highly significantly increased in two of the ponies.

The possible physiological mechanisms effecting these changes are discussed.

SECTION No.4

A STUDY OF SOME ASPECTS OF METABOLIC ACIDOSIS FOLLOWING AMMONIUM CHLORIDE ADMINISTRATION TO PONIES

INTRODUCTION

Although the state of metabolic acidosis in man has been extensively studied, no reference to a thorough investigation of this disturbance in equine species was discovered. It is customary to base judgement of normal or abnormal acid/base status upon the pH of the blood[53]. A state of acidosis is deemed to exist when the pH of blood falls below the accepted lower limit of normality[53]. Acidosis may arise due to respiratory or non-respiratory causes, and in the latter case is commonly termed metabolic acidosis. In metabolic acidosis an excess of acid and/or a deficit of base is present[53].

The normal blood pH range has been well established in man[53 105 169], but the same does not appear to be true for equine species. Tables No. 8, 9 and 10 in Section No.1 of this thesis summarise the results obtained by other workers who have investigated equine blood acid-base parameters, but it is evident from these results that large variations have been detected, such that no consistent ranges of normal values appear to have been widely adopted. By the standard of human values some of the results quoted in Table No.8 would be considered frankly acidotic.

Few authors whose results are quoted in Tables 8, 9 and 10 commented upon the significance of the results they obtained, though Littlejohn[52] proposed that a pCO_2 of 44 mmHg be adopted as the normal mean venous pCO_2 of horses, as opposed to the oft-quoted human mean pCO_2 of 40 mmHg.

Because so many different methods of measuring bicarbonate concentrations are in common use the comparison of various bicarbonate concentrations published is made difficult, if not impossible.

No thorough study of the state of metabolic acidosis induced by the administration of acidifying agent to horses appears to have been reported, though two publications[89 170] give details of the effect upon equine urine of the oral administration of acidifying salts. Piperno, Ellis, Getty and Brody[170] gave ammonium chloride at a dose rate of 60gs/1000lbs (453.6 kg) body weight every four hours for approximately 60 hours, and they observed a reduction of 3 pH units in urine. Nicholson[89] investigated the effects of sodium dihydrogen orthophosphate (100 g), calcium chloride hexahydrate (100g) and ammonium chloride (2 x 50g) upon the pH of the urine of small ponies. He discovered that ammonium chloride lowered the pH of the urine by approximately 2 pH units, whereas sodium dihydrogen orthophosphate produced no significant effect and calcium chloride hexahydrate only decreased urinary pH by circa one unit. None of these authors measured blood acid-base parameters.

Metabolic acidosis was induced in Shetland and Shetland cross ponies in order to observe changes in blood pH, pCO_2 and serum bicarbonate concentration, and also to study concurrent changes in selected blood and urine constituents. The choice of the acidifying salt administered to induce acidosis was based upon the work of Nicholson[89], and ammonium

chloride was duly selected. Because Nicholson[89] had administered 100g over a six hour period it was anticipated that 50g of ammonium chloride daily for seven consecutive days would be adequate to induce acidosis without incurring any risk to the animal's life. This judgement was erroneous, as will become evident at a later stage, and for the second part of the experimental work a daily dose of 40g of ammonium chloride was administered for five consecutive days.

METHODS

The experimental work was conducted in two separate parts, owing to a blood gas analyser not being available initially. The basic management of the ponies has already been described.

During the first part of the work five Shetland and Shetland cross ponies were studied, but one of the ponies died during the course of an experiment.

Throughout the times that urine was collected[94] the ponies were confined to small stalls. At other times they were housed in looseboxes. 50g of ammonium chloride in aqueous solution was administered via a stomach tube for each of seven consecutive days. Immediately before the first dose was administered a blood sample was withdrawn from the jugular vein (see Section No.1, Part 1). After administration of the acidifying salt urine collection commenced.

Blood sampling and ammonium chloride administration were undertaken at 10.00 hours. Blood sampling always preceded ammonium chloride administration. Hence, subsequent blood samples were collected at the end of the 24 hour period following ammonium chloride administration. After Day 7 blood samples were only collected on alternate days, and after Day 11 one final sample was collected on Day 14. The daily food ration was supplied at 16.00 hours, and drinking water was available ad libitum throughout the entire experimental period.

The techniques of blood collection and analyses have

already been described, (see Section No.1, Part 1). Packed cell volume percentage, and plasma sodium, potassium, chloride, inorganic phosphate and urea concentrations were measured in this part of the experiment. The ponies were permitted to stand down from the stalls on Day 5 because seven or eight days was considered too long a time to stand in the very close confines of the stall. They were taken to looseboxes as soon as they had received the acidifying salt, and were returned to the stalls the following day, before the next dose of ammonium chloride was given.

Urine volume, pH, specific gravity and the sodium, potassium, chloride, inorganic phosphate, ammonium and urea content were measured using techniques described in Section No.1, Part 2, of this thesis.

The second part of the experiment involved the study of four ponies. In view of the fact that the pony who died had a unilateral parotid fistula it was decided to exclude the other fistulated pony. This was in order to avoid administering ammonium chloride to a pony who might be abnormally susceptible to the effect of this acidifying agent owing to the loss of bicarbonate in saliva excreted from the fistulated duct[55]. Accordingly, four non-fistulated ponies were studied, three of which had been studied in the first half of the work. In a further effort to safeguard the animals from a fatal outcome the dose of ammonium chloride was reduced to 40g daily for each of five consecutive days.

The timing of blood sampling, ammonium chloride administration, urine collection and feeding was identical with that adopted for the first part of the study, but the duration of the experiment was shortened not only by administering ammonium chloride for five days instead of seven, but reducing the "follow up" period to four days instead of seven. As before, the ponies were allowed to stand down from the stalls on Day 5.

Blood samples were subjected to pH and pCO_2 determinations, and serum bicarbonate concentrations were determined. The analytical methods employed were those described in Section No.1, Part 1.

The volume and pH of urine samples were measured, and the net acid/base content was determined (see Section No.1, Part 2).

RESULTS

Because the number of ponies studied was small, and to avoid masking individual responses to ammonium chloride administration, the results from each pony are presented singly. The significance of the changes observed in the parameters examined is based upon the mean value and standard deviation for the parameters in the untreated individual (see Section No.1, Parts 1 and 2). A value outwith the normal mean $\pm$ 2 standard deviations was considered statistically significant ($p < 0.05$) and a value outwith the normal mean $\pm$ 3 standard deviations was considered highly significant ($p < 0.01$).

During the first part of the experiment all the ponies showed some distress following the ingestion of ammonium chloride. Varying degrees of sweating, paddling movements of the limbs, increases in the respiratory rate, and dilation of the pupils were observed. Occasionally the ponies were noticed to stare and bite at their flanks. Towards the end of the week during which ammonium chloride was ingested, all ponies were observed to refuse part of the daily hay ration.

The death of one pony on the 9th day of the first experiment was attributed to irreversible metabolic acidosis. The results obtained from the analyses of blood and urine samples from this pony over the preceding 8 days were excluded from the study because it was believed that some were influenced by parenteral therapy administered in an effort to correct the acidosis.

During the second part of the experiment three of the four ponies showed only mild transient distress. However, the fourth pony (MacGowan) was severely affected. Pronounced muscular tremors, profuse sweating and signs suggestive of colic were observed. Hence, ammonium chloride administration to this pony was abandoned on the third day. He was returned to a loosebox and spontaneous recovery occurred.

The results obtained from both parts of the experiment are presented in Tables 77 to 97.

KEY TO THE SYMBOLS USED IN TABLES 77 TO 97

* Denotes a significant increase ($p < 0.05$)
\+ Denotes a highly significant increase ($p < 0.01$)
$\not$ Denotes a significant decrease ($p < 0.05$)
x Denotes a highly significant decrease ($p < 0.01$).

TABLE No.77

THE EFFECT OF AMMONIUM CHLORIDE UPON BLOOD pH.

		NH_4Cl Administration	Jimmie	Billie	Ben	MacGowan
Before NH_4Cl		none	7.410	7.415	7.405	7.405
Day	1	40g	7.360	7.400	7.395	7.380
"	2	40g	7.370	7.370	7.405	7.390
"	3	40g	7.350$^{\not}$	7.360	7.380	-
"	4	40g	7.280^{x}	7.350	7.360	-
"	5	40g	7.335^{x}	7.315$^{\not}$	7.355	-
"	6	none	7.350$^{\not}$	7.380	7.375	-
"	7	none	7.375	7.450	7.410	-
"	8	none	7.415	7.430	7.435	-
"	9	none	7.420	7.410	7.430	-

Marked decreases in blood pH were observed in two of the three ponies who completed the experiment. It was noted that of these ponies, the one who appeared most distressed after receiving ammonium chloride incurred the greatest decreases in blood pH. The third pony also exhibited lowered blood pH values, but the decreases were not statistically significant ($p \geq 0.05$).

TABLE No.78

THE EFFECT OF AMMONIUM CHLORIDE UPON BLOOD pCO_2 (mmHg)

		NH_4Cl Administration	Jimmie	Billie	Ben	MacGowan
Before NH_4Cl		none	42.0	44.0	44.5	39.5
Day	1	40g	45.0	46.0	47.0	43.5
"	2	40g	47.0	38.0^{x}	35.5^{x}	40.0
"	3	40g	44.0	45.0	46.5	-
"	4	40g	43.0	39.5$^{\prime}$	38.0$^{\prime}$	-
"	5	40g	41.0	42.0	38.0$^{\prime}$	-
"	6	none	43.5	50.0	48.0	-
"	7	none	40.0	43.0	47.5	-
"	8	none	39.0$^{\prime}$	43.5	39.5	-
"	9	none	45.5	43.5	42.0	-

Decreases in blood pCO_2 were observed in all three ponies who completed the experiment. The pony who exhibited the greatest decreases in pCO_2 showed no significant decrease in blood pH. One pony exhibited an abnormally low pCO_2 on one occasion only, three days after receiving the last dose of ammonium chloride.

TABLE No.79

THE EFFECT OF AMMONIUM CHLORIDE UPON SERUM BICARBONATE CONCENTRATION (mEq/l)

		NH_4Cl Administration	Jimmie	Billie	Ben	MacGowan
Before	NH_4Cl	none	25.6	27.7	26.5	23.9^{x}
Day	1	40g	$24.3^{\prime}$	27.7	27.7	26.6
"	2	40g	23.7^{x}	22.2^{x}	$21.5^{\prime}$	$25.4^{\prime}$
"	3	40g	22.4^{x}	24.5^{x}	26.7	-
"	4	40g	18.4^{x}	20.9^{x}	23.3	-
"	5	40g	21.2^{x}	20.9^{x}	20.5^{x}	-
"	6	none	22.6^{x}	28.3	27.3	-
"	7	none	25.7	28.7	28.6	-
"	8	none	25.5	27.9	28.3	-
"	9	none	28.6	27.6	26.4	-

Highly significant decreases ($p < 0.01$) in serum bicarbonate were observed in all ponies, though the onset of these decreases did not always occur immediately, and neither were they invariably sustained. The maximum decreases in the serum bicarbonate concentrations of the ponies who each received the five daily doses of ammonium chloride were almost identical in magnitude. The pony who was withdrawn from the experiment exhibited an abnormally low serum bicarbonate concentration before he received any ammonium chloride.

TABLE No.80

THE EFFECT OF AMMONIUM CHLORIDE UPON PACKED CELL VOLUME PERCENTAGE

		NH_4Cl Administration	Scruffy	Jimmie	Billie	Ben
Before	NH_4Cl	none	34.0	28.5	35.0	36.0
Day	1	50g	37.0	26.5	33.0	37.0
"	2	50g	32.0	26.0*	35.0	37.5
"	3	50g	35.5	26.0*	35.0	37.5
"	4	50g	35.5	25.0*	31.5	34.5
"	5	50g	33.5	24.0*	37.0	34.0
"	6	50g	35.5	26.0*	38.0	38.0
"	7	50g	35.5	25.5*	35.5	34.5
"	9	none	32.0	25.5*	32.5	31.0
"	11	none	33.0	24.5*	32.5	32.5
"	14	none	32.0	25.5*	34.5	34.0

Though the packed cell volume percentage of one pony (Jimmie) was abnormally low from day 2 onwards the differences between these values and the one obtained immediately before ammonium chloride ingestion began were small.

TABLE No.81

THE EFFECT OF AMMONIUM CHLORIDE UPON PLASMA SODIUM CONCENTRATION (mEq/l)

		NH_4Cl Administration	Scruffy	Jimmie	Billie	Ben
Before NH_4Cl		none	133	135	133	130
Day	1	50g	133	133	125*	128
"	2	50g	133	128	131	133
2	3	50g	128	130	128	130
"	4	50g	133	133	130	130
"	5	50g	133	134	133	133
"	6	50g	130	123*	128	128
"	7	50g	130	123*	125*	130
"	9	none	133	130	130	127
"	11	none	133	130	125*	127
"	14	none	133	130	133	129

Transient falls in plasma sodium concentration were observed in two of the four ponies studied.

TABLE No.82

THE EFFECT OF AMMONIUM CHLORIDE UPON PLASMA POTASSIUM CONCENTRATION (mEq/l)

		NH_4Cl Administration	Scruffy	Jimmie	Billie	Ben
Before	NH_4Cl	none	4.20	4.50	4.10	3.40
Day	1	50g	2.10[x]	4.40	4.20	3.90
"	2	50g	3.40	4.50	3.50	3.80
"	3	50g	4.30	3.80	3.40	2.60[/]
"	4	50g	4.10	4.00	3.40	4.30
"	5	50g	4.00	4.20	4.30	2.80
"	6	50g	2.30[/]	4.00	4.50	2.30[/]
"	7	50g	4.10	4.40	3.60	3.90
"	9	none	4.40	4.40	4.40	3.20
"	11	none	3.20	4.60	3.70	3.50
"	14	none	3.00	5.00	4.30	3.90

The plasma potassium concentration of two ponies exhibited transient decreases during the period of ammonium chloride ingestion

TABLE No.83

THE EFFECT OF AMMONIUM CHLORIDE UPON PLASMA CHLORIDE CONCENTRATION (mEq/l)

		NH_4Cl Administration	Scruffy	Jimmie	Billie	Ben
Before	NH_4Cl	none	92[x]	96	101	99
Day	1	50g	92[x]	99	98	98
"	2	50g	95[/]	102	103	100
"	3	50g	94[/]	98	102	98
"	4	50g	106[*]	101	104	101
"	5	50g	100	101	100	104
"	6	50g	103	104	98	103
"	7	50g	99	103	106[*]	99
"	9	none	95[/]	94	98	99
"	11	none	95[/]	98	100	101
"	14	none	102	98	104	97

The plasma chloride concentrations of two ponies exhibited significant but dissimilar changes. A significant increase in the plasma chloride concentration of one pony (Billie) was observed on day 7. The other pony (Scruffy) commenced the experiment with an abnormally low plasma chloride concentration, which increased until, on day 4, it was abnormally high. Thereafter the plasma chloride concentration decreased until, on days 9 and 11, it was abnormally low again. At the conclusion of the experiment the level of this plasma constituent was normal.

TABLE No.84

THE EFFECT OF AMMONIUM CHLORIDE UPON PLASMA INORGANIC PHOSPHATE CONCENTRATION (mgP/100ml)

		NH_4Cl Administration	Scruffy	Jimmie	Billie	Ben
Before NH_4Cl		none	3.98	3.13	2.93	3.47
Day	1	50g	2.85	3.05	2.40	3.12
"	2	50g	2.29	2.73	2.38	3.21
"	3	50g	2.61	3.18	3.52	3.05
"	4	50g	2.31	3.28	3.14	3.00
"	5	50g	3.00	2.99	2.58	3.45
"	6	50g	3.40	3.02	3.52	2.95
"	7	50g	3.63	2.09	3.83	3.39
"	9	none	3.09	3.23	0.99[x]	3.04
"	11	none	3.63	4.14	4.48[*]	2.77
"	14	none	2.59	3.37	5.37[+]	3.86

Changes in the plasma inorganic phosphate concentrations of one pony were observed but these changes all occurred after the cessation of the ammonium chloride ingestion.

TABLE No.85

THE EFFECT OF AMMONIUM CHLORIDE UPON PLASMA UREA CONCENTRATION (mg urea/100 ml)

		NH_4Cl Administration	Scruffy	Jimmie	Billie	Ben
Before NH_4Cl		none	33.5	107.0+	34.0	35.5
Day	1	50g	50.2*	107.0+	30.5	48.9
"	2	50g	44.6	82.8*	30.5	53.3*
"	3	50g	72.5+	49.4	30.1	55.9*
"	4	50g	62.8+	78.2	28.2	53.3*
"	5	50g	54.4+	111.0+	37.7	75.8+
"	6	50g	48.7*	111.0+	37.5	57.9*
"	7	50g	26.4	145.0+	49.4	39.5
"	9	none	20.8	93.8+	29.1	38.8
"	11	none	20.8	60.8	24.3	45.4
"	14	none	22.2	60.3	24.5	29.0

Three ponies exhibited large increases in plasma urea concentrations. Although one of the three (Jimmie) commenced the experiment with an abnormally high plasma urea level, further increases were observed during days 5, 6 and 7, and the plasma urea concentration of this pony did not return to within normal limits until after day 9.

Elevations in the plasma urea concentration of these ponies showed no close correlation with the urinary urea content, and neither did the times of maximum plasma urea concentrations coincide with those of maximum urinary loss.

TABLE No.86

THE EFFECT OF AMMONIUM CHLORIDE UPON URINE VOLUME/24 HOURS

(ml)

		NH_4Cl Administration	Scruffy	Jimmie	Billie	Ben
Day	1	50g	5510	5270	3960	6160
"	2	50g	6960	4330	4220	3670
"	3	50g	4250	4110	2850	3820
"	4	50g	3930	5300	4280	4280
"	5	50g	——— Urine not collected ———			
"	6	50g	5210	4000	3010	3480
"	7	50g	6490	5160	2830	4460
"	8	none	5910	2490	2410	2310
"	10	none	3990	3110	1460	2370
"	12	none	4660	2750	560 ≠	2880
"	14	none	3420	3890	2300	2750

During the period of daily administration of 50g of ammonium chloride no changes in the 24 hour urine volumes were observed.

TABLE No.87

THE EFFECT OF AMMONIUM CHLORIDE UPON URINE VOLUME/24 HOURS

(ml)

		NH_4Cl Administration	Jimmie	Billie	Ben	MacGowan
Day	1	40g	6880*	5550	8460+	5480
"	2	40g	5060	4510	8270+	6360
"	3	40g	5150	3420	5630	-
"	4	40g	5420	5230	7170*	-
"	5	40g	——— Urine not collected ———			
"	6	none	3670	3580	3860	-
"	8	none	3140	4290	4680	-
"	10	none	2240	4340	3990	-

During the ingestion of 40g of ammonium chloride/day the urine volume voided by Jimmie on day 1 and the urine volumes voided by Ben on days 1, 2 and 4 were markedly increased above normal.

TABLE No.88

THE EFFECT OF AMMONIUM CHLORIDE UPON URING SPECIFIC GRAVITY

		NH_4Cl Administration	Scruffy	Jimmie	Billie	Ben
Day	1	50g	1.016	1.016[x]	1.029	1.016
"	2	50g	1.013	1.026	1.022	1.028
"	3	50g	1.021	1.024	1.030	1.027
"	4	50g	1.019	1.017[x]	1.023	1.026
"	5	50g	——— Urine not collected ———			
"	6	50g	1.018	1.023	1.021	1.027
"	7	50g	1.016	1.016[x]	1.030	1.022
"	8	none	1.011	1.028	1.029	1.037
"	10	none	1.023	1.032	1.042	1.037
"	12	none	1.021	1.026	1.039	1.038
"	14	none	1.033	1.027	1.034	1.042

Decreases were observed in the specific gravity of the urine voided by Jimmie on days 1, 4 and 7. The decreases corresponded with the three greatest volumes of urine voided/24 hours by this pony during this first part of the experiment.

TABLE No.89

THE EFFECT OF AMMONIUM CHLORIDE UPON URINE pH

		NH_4Cl Administration	Scruffy	Jimmie	Billie	Ben
Day	1	50g	9.00	9.05	7.20✓	8.95
"	2	50g	8.60	7.45✓	8.15	7.15✓
"	3	50g	8.75	8.40	8.00	7.40✓
"	4	50g	8.80	8.45	8.10	7.30✓
"	5	50g	——— Urine not collected ———			
"	6	50g	8.55	6.75x	8.30	7.85
"	7	50g	8.00	9.10	7.25✓	7.05x
"	8	none	8.45	6.35x	7.90	7.25✓
"	10	none	8.75	8.45	8.90	8.80
"	12	none	7.75	8.90	8.90	8.95
"	14	none	8.65	8.85	8.95	8.80

The pH of all urine samples collected during both parts of the experiment was measured. During the first part of the experiment the urine samples from one pony showed no significant changes in pH. The pH of urine samples voided by the other three ponies was decreased at various times during days 1 to 8, and all three produced acid urine on two or more days.

TABLE No.90

THE EFFECT OF AMMONIUM CHLORIDE UPON URINE pH

		NH_4Cl Administration	Jimmie	Billie	Ben	MacGowan
Day	1	40g	8.65	8.20	7.35$^{\checkmark}$	7.70
"	2	40g	7.55	7.15$^{\checkmark}$	7.20$^{\checkmark}$	7.65
"	3	40g	8.45	7.70	6.55^{x}	-
"	4	40g	7.15$^{\checkmark}$	5.85^{x}	6.90^{x}	-
"	5	40g	—— Urine not collected ——			
"	6	none	6.90^{x}	7.70	6.80^{x}	-
"	8	none	8.45	8.20	8.65	-
"	10	none	8.65	8.15	8.80	-

During the daily administration of 40g of ammonium chloride changes in urine pH were similar to those observed when a higher dose of ammonium chloride was ingested.

TABLE No.91

THE EFFECT OF AMMONIUM CHLORIDE UPON THE NET ACID/BASE CONTENT OF URINE (mEq/24 hours)

		NH_4Cl Administration	Jimmie	Billie	Ben	MacGowan
Day	1	40g	105	50$^{\checkmark}$	55$^{\checkmark}$	12
"	2	40g	-56$^{\checkmark}$	28^{x}	-108^{x}	-30
"	3	40g	-118^{x}	-3^{x}	-673^{x}	-
"	4	40g	-137^{x}	-52^{x}	-43^{x}	-
"	5	40g	—— Urine not collected ——			
"	6	none	-75$^{\checkmark}$	183	45$^{\checkmark}$	-
"	8	none	-5$^{\checkmark}$	228	287	-
"	10	none	294	411	446	-

N.B. - Denotes the excretion of net acid.

The pattern of renal net acid/base excretion was similar in all ponies. Following the first dose of ammonium chloride the quantity of base excreted was low. After receiving the second dose of ammonium chloride three ponies excreted acid, whilst the fourth exhibited a further reduction in base excretion. From day 3 the three ponies who continued the experiment all excreted net acid until ingestion of the salt ceased. Though one pony excreted acid on days 6 and 8, the other two reverted to base excretion on day 6 onwards. It was noted that renal net acid excretion occurred on some occasions when the urine pH was alkaline, and conversely net base was sometimes excreted in urine where the pH was acidic. When acid was added to aliquots of urine which had been voided after the ponies had received ammonium chloride little or no frothing was observed. It was assumed that this indicated either a great reduction in, or the absence of, bicarbonate[95].

TABLE No.92

THE EFFECT OF AMMONIUM CHLORIDE UPON THE SODIUM CONTENT OF URINE (mEq/24 hours)

		NH_4Cl Administration	Scruffy	Jimmie	Billie	Ben
Day	1	50g	187	501+	319+	728+
"	2	50g	557+	165	422+	349*
"	3	50g	790+	144	254+	254
"	4	50g	197	424+	526+	342*
"	5	50g	——— Urine not collected ———			
"	6	50g	443+	192	211	331*
"	7	50g	344*	377+	65	223
"	8	none	30	37	24	32
"	10	none	32	39	15/	71
"	12	none	182	63	6/	78
"	14	none	41	324*	41	63

Large increases were observed in the sodium content of the urine voided by the ponies on many, but not all, the days during the time that ammonium chloride was ingested. Immediately ammonium chloride ingestion ceased urinary sodium decreased. Following this rapid decrease urinary sodium remained low for varying periods of time, but it had begun to increase by the end of the experiment. The sodium content of the final urine sample obtained from one pony (Jimmie) was abnormally high.

TABLE No.93

THE EFFECT OF AMMONIUM CHLORIDE UPON THE POTASSIUM CONTENT OF URINE (mEq/24 hours)

		NH_4Cl Administration	Scruffy	Jimmie	Billie	Ben
Day	1	50g	937	843	990	710
"	2	50g	835	1212	675	881
"	3	50g	978	1069	713	850
"	4	50g	629	901	685	792
"	5	50g	—— Urine not collected ——			
"	6	50g	782	920	482	760
"	7	50g	779	851	736	847
"	8	none	590	685	506	739
"	10	none	998	1306	642	853
"	12	none	932	935	213	1049
"	14	none	787	615	805	1375

No significant changes in the urinary potassium content were observed during ammonium chloride administration. The low result obtained from Billie on day 12 coincided with a very small volume of urine voided.

TABLE No.94

THE EFFECT OF AMMONIUM CHLORIDE UPON THE CHLORIDE CONTENT OF URINE (mEq/24 hours)

		NH_4Cl Administration	Scruffy	Jimmie	Billie	Ben
Day	1	50g	1030	1173^{+}	1338^{+}	1023
"	2	50g	1357*	1398^{+}	1312^{+}	1341*
"	3	50g	1136*	1344^{+}	1217^{+}	1249
"	4	50g	845	1367^{+}	1421^{+}	1387^{+}
"	5	50g	——— Urine not collected ———			
"	6	50g	1274*	1180^{+}	924	1632^{+}
"	7	50g	1525^{+}	1690^{+}	976	732
"	8	none	1448^{+}	754	610	816
"	10	none	451	675	279	590
"	12	none	622	418	115^{+}	445
"	14	none	363	568	313	635

The ponies excreted very large quantities of chloride on most of the days on which they received ammonium chloride. After day 7 the chloride content of all 24 hour urine samples was normal, with the exceptions of the sample voided by Scruffy on day 8, and by Billie on day 12. The reduced chloride excretion by Billie coincided with an abnormally low volume of urine voided.

TABLE No.95

THE EFFECT OF AMMONIUM CHLORIDE UPON THE INORGANIC PHOSPHATE CONTENT OF URINE (mgP/24 hours)

		NH_4Cl Administration	Scruffy	Jimmie	Billie	Ben
Day	1	50g	87.2	112.1	150.5	397.3
"	2	50g	33.4	297.6	117.5	966.4+
"	3	50g	42.0	70.9	197.5+	894.4+
"	4	50g	194.4+	65.7	515.7+	884.1*
"	5	50g	——— Urine not collected ———			
"	6	50g	459.2+	596.8*	108.9	818.7*
"	7	50g	368.1+	114.9	622.6+	1040.6+
"	8	none	38.7	328.5	438.6+	706.9*
"	10	none	50.4	53.1	66.4	633.0
"	12	none	151.7*	121.8	21.2	156.5
"	14	none	40.2	115.4	109.6	141.0

Urinary inorganic phosphate excretion was increased at various times during ammonium chloride ingestion. Two ponies also excreted unusually large amounts of inorganic phosphate on day 8, and another pony likewise excreted an excessive quantity of inorganic phosphate on day 12, but a general trend towards a return to normal when ammonium chloride administration ceased was evident.

TABLE No.96

THE EFFECT OF AMMONIUM CHLORIDE UPON THE AMMONIUM CONTENT OF URINE (mEq/24 hours)

		NH_4Cl Administration	Scruffy	Jimmie	Billie	Ben
Day	1	50g	591+	485*	144	719+
"	2	50g	431	296	223	329*
"	3	50g	536*	529*	212	384*
"	4	50g	356	358	420	380
"	5	50g	——— Urine not collected ———			
"	6	50g	421	238	462	610+
"	7	50g	287	701+	294	483*
"	8	none	300	204	320	224
"	10	none	218	222	214	313
"	12	none	191	220	70	390
"	14	none	240	234	262	285

Three ponies exhibited marked increases in urinary ammonium excretion during the time they received ammonium chloride. Within 24 hours of the cessation of ammonium chloride ingestion the urinary ammonium content returned to within normal limits even when urine pH remained low.

TABLE No.97

THE EFFECT OF AMMONIUM CHLORIDE UPON THE UREA CONTENT OF URINE (g urea/24 hours)

	NH_4Cl Administration	Scruffy	Jimmie	Billie	Ben
Day 1	50g	25.3	30.9	52.0	16.3
" 2	50g	34.6	54.1	43.9	46.1
" 3	50g	35.6	43.0	39.8	49.0
" 4	50g	55.8+	47.1	48.3	50.7
" 5	50g	——— Urine not collected ———			
" 6	50g	43.5*	44.9	30.3	52.5
" 7	50g	40.4	32.9	59.7*	45.9
" 8	none	28.0	31.7	55.9	36.5
" 10	none	22.4	30.3	22.0	31.0
" 12	none	27.4	13.1	6.4+	30.4
" 14	none	28.0	12.8	19.0	22.8

All the ponies excreted large quantities of urea during ammonium chloride ingestion, but the increases were only significant in two ponies. The unusually small quantity of urea excreted by Billie on Day 12 coincided with an abnormally low 24 hour urine volume.

DISCUSSION

A judgement of acidosis is normally based solely upon the pH of blood[53], though plasma or serum bicarbonate concentrations and pCO_2 also show characteristic changes during acid/base disturbances. Because the normal range of equine venous blood pH does not appear to have been satisfactorily established, judgement of normality or abnormality was based upon the values of the acid/base parameters measured in the untreated ponies (see Section No.1 Part 1, Tables 19,20 and 21).

From the results of the blood pH determinations it was concluded that ammonium chloride, administered at a dose rate of 40g daily for five days, induced varying degrees of metabolic acidosis in these ponies. Respiratory compensation[53], typified by decreases in pCO_2 concurrently with reduced plasma bicarbonate concentrations, minimised the pH changes, and in the case of one pony (Ben) was effective in maintaining blood pH within normal limits throughout the investigation. The pony who exhibited no significant decreases in pCO_2 during days 1 to 5 showed the most severe acidosis.

In addition to the bicarbonate/carbonic acid buffer system other blood buffers were probably involved[53 171]. The phosphate buffer system will be discussed at a later stage. Had a blood gas analyser been available when 50g of ammonium chloride was given for seven days, acid-base parameters could have been investigated simultaneously with plasma electrolytes, which would have made more meaningful

comparisons possible. Failing this identical doses of ammonium chloride in each experiment would have been preferable, but for reasons already stated this was considered unsafe. Haemoglobin and plasma proteins were also believed to contribute substantially to blood buffering of the acid load[171].

One pony (Jimmie) began the experiment with a low packed cell volume percentage, and although further decreases were observed fluctuations were not substantially greater than those observed in the packed cell volume percentages of the other ponies. Hence it was concluded that no significant changes which could be attributed to ammonium chloride ingestion occurred in the packed cell volume percentage of these ponies.

Despite heavy urinary sodium losses during the times the ponies were receiving ammonium chloride, there were few decreases in plasma sodium concentrations. The decreased plasma sodium concentration which was observed after the administration of the first 50g of ammonium chloride to one pony (Billie) may have indicated initial difficulty in plasma sodium regulation following a very large urinary sodium loss. Because the plasma sodium levels of two ponies showed no change, and only intermittent changes were observed in the other two ponies, it was concluded that sodium concentrations in plasma were maintained at the expense of sources elsewhere in the body.

Two sources of sodium which might have been drawn upon are bone and intestinal fluid[23 172]. Though the sodium

content of the faecal fluids of these ponies was shown to be low (see Section No.1, Part 3) Alexander[23] demonstrated that the liquor in the equine small intestine and caecum had a sodium concentration only marginally lower than that in the plasma of these ponies. In view of the volume of the equine digestive tract[93], this represents a considerable quantity of sodium. Moreover, Burnell and Teubner[172] showed that the sodium content of tibial cortical bone of dogs decreased when the dogs were maintained over 5-10 days in a state of metabolic acidosis. If tibial cortical bone typifies that from other sites, and if this decrease also occurs in equine animals, then bone and intestinal liquor are two likely sources of sodium under conditions of negative sodium balance. Faecal fluid analyses were not undertaken, but since most of the sodium in the intestinal tract is absorbed cranially to the rectum[23] the results of such analyses may not have been especially valuable. The sharp fall in urinary sodium following the cessation of ammonium chloride ingestion suggested that the body reserves of sodium were being restored.

No reference to the effect of metabolic acidosis upon plasma potassium concentrations in the horse was discovered, but metabolic acidosis in man and dogs is usually associated with an elevated plasma potassium level[53 173 174 175 176 177]. When dogs were used in acute experiments hyperkalaemia was noted as blood pH decreased[174 175 176] regardless of whether the acidosis was metabolic or respiratory in origin. The rise in plasma potassium concentrations was followed by an

increased urinary output[175]. Simmons and Avedon[177], using dogs, were able to demonstrate that a decrease of one pH unit in blood was accompanied by a rise of 3.0 to 5.0 mEq/l in the plasma potassium level. However, fluctuations in serum (and presumably plasma) potassium concentrations do not necessarily indicate changes in total body potassium, since potassium is chiefly an intracellular ion[53].

The equine diet is potassium rich[97] and urinary potassium represents the excretion of excess[86], so the possibility of a dietary potassium deficiency was disregarded as a cause of the failure of the plasma potassium concentrations of the ponies to increase during acidosis. Because only transient decreases in the plasma potassium concentrations were observed in two of the four ponies studied it was concluded that ammonium chloride ingestion and the resulting acidosis had no profound effect upon the plasma potassium levels of these ponies. However it is not known whether the failure of the potassium levels to rise was a feature of the equine species or whether it was peculiar to this group of ponies. It was not considered safe to increase the dose of ammonium chloride for further investigations.

The overall lack of significant elevations in plasma chloride concentration was unexpected[53], both in view of the clinical and biochemical signs indicative of metabolic acidosis, and in view of the fact that a high dose of ammonium chloride was administered. The increase in urinary

chloride was considered evidence of the absorption of a large part of the dose,(50g of ammonium chloride contains 935 mEq of chloride), but obviously these ponies were able to regulate chloride absorption in such a manner that the plasma chloride concentration almost invariably remained within normal limits.

It was not known why Scruffy exhibited large fluctuations in plasma chloride concentration, which was abnormally low at the commencement of the experiment. Doubtlessly the ingestion of ammonium chloride caused the sudden increase observed on day 4 but it is difficult to understand why this increase did not occur at an earlier stage in the experiment. The reason for the decrease below normal of the plasma chloride concentration during days 9 and 11 was not understood. It was unfortunate that serum bicarbonate concentrations were not measured concurrently with plasma chloride concentrations as this would have indicated whether or not reciprocal variations in bicarbonate occurred[178 179].

The ingestion of 50g of ammonium chloride for seven consecutive days produced no changes in the plasma inorganic phosphate concentrations of these ponies during this time. However, since phosphate is principally an intracellular ion[53], it is possible that changes in intracellular concentrations occurred which were not reflected by simultaneous fluctuations in plasma levels. Doubtlessly plasma inorganic phosphate was involved in the buffering of the acid load imposed upon the ponies by ammonium chloride assimilation, but since buffering by phosphate is effected by changes in

the proportion of mono- and di-hydrogen phosphate ions[53] [106] this alone would not change the concentration of phosphorus.

The abnormal levels of plasma inorganic phosphate in Billie on days 9, 11 and 14 could have arisen due either to a degree of phosphate depletion followed by repletion or a disturbance in phosphate movement into plasma. Though the plasma levels were maintained within normal limits during ammonium chloride ingestion, increased urinary inorganic phosphate excretion continued after ammonium chloride ingestion ceased. It is possible that the low plasma concentration on day 9 resulted from the previous large phosphate loss in urine. When urinary loss decreased sharply on day 10 the very pronounced increase in plasma inorganic phosphate could have been caused by an abrupt swing from negative to positive phosphate balance. It appeared that normal phosphate balance had still not been attained by this pony by day 14. Nevertheless, it was concluded that these ponies were capable of maintaining normal plasma inorganic phosphate concentrations during the period of ammonium chloride ingestion, despite large increases in urinary phosphate excretion.

The increases in the concentrations of plasma urea shown by three of the four ponies during the time they received ammonium chloride were attributed to the conversion to urea of the ammonia component of the salt and the subsequent circulation of the urea so formed[53]. One of the ponies began the experiment with an abnormally high plasma

urea level, for which no explanation is forthcoming. Nevertheless the plasma urea concentration of this pony increased further throughout the course of ammonium chloride administration.

One of the four ponies failed to show any significant increase in plasma urea concentration. It was believed that this could be due to a more rapid metabolism of ammonium chloride by this pony, and a faster excretion of the excess urea produced. Thus, by the time the blood sample was collected twenty-four hours after ammonium chloride administration, the blood urea concentration would have reverted to a normal level. From the clinical signs exhibited by the ponies ammonium chloride was judged to exert its maximum effect between approximately 1 to 4 hours after ingestion. Had blood sampling been undertaken during this time it is probable that greater changes in plasma urea concentrations than those detected in samples collected 24 hours after administration of the salt would have been detected.

Because the volumes of urine voided by the ponies were unaffected by ammonium chloride at the higher dose rate, and since only two ponies produced significantly more urine/24 hours on a few occasions after receiving lower doses of ammonium chloride, it was concluded that diuresis was not a major effect of ammonium chloride ingestion by these ponies. Nevertheless these ponies were able to excrete very large quantities of sodium and chloride despite no consistent substantial increases in urine volume. Thus

the ponies differed from man in their response to this substance[53].

Specific gravity measurements were only performed upon the urine produced following the administration of ammonium chloride at the higher dose rate, and only one pony showed evidence of significant decreases in urinary specific gravity. These decreases were observed in the twenty-four hour urine volumes which, though not significantly elevated, were higher than the pony customarily produced. This accords with the observation (see Section No.1, Part 2) that the higher the volume of urine produced, generally, the lower the specific gravity[89].

In the context of this work urine pH was considered acid when it was lower than blood pH, and alkaline when it was greater than blood pH. Three ponies received ammonium chloride at both dose rates and all voided urine with significantly lower pH values, though not all 24 hour samples exhibited this pH fall, and not all the abnormally low pH values were actually acid. The pony who was withdrawn from the second part of the experiment showed a trend towards a decreased urine pH, though the decreases were not statistically significant. The pH of the urine voided by Scruffy showed virtually no change throughout the time he received ammonium chloride and the reason for this pony differing from the others is unknown. However, this pony might have excreted acid via the parotid salivary duct fistula.

The results listed in Tables 90 and 91, showed that

net acid was excreted on some days when the urine pH was alkaline, and net base on other days when the urine pH was acid. It was concluded, therefore, that the acid base status of the urine was a better indication of the acid base status of the animal, and the renal response to acid loading, than the urinary pH value, which is greatly influenced by the urine buffers[53 105 106].

The evidence for a degree of respiratory compensation occurring in addition to renal acid excretion[53 171] has already been presented. Had the acid load been excreted solely via the kidneys greater quantities of acid in the urine would have been expected.

From the very large increases in urinary sodium excretion observed during days 1 to 7 it was concluded that ammonium chloride was effective in inducing natriuresis. Furthermore, natriuresis occurred without a concurrent diuresis. Variations in the time and frequency of micturition could have been partly responsible for the fact that not every urine sample contained abnormally large amounts of sodium. It was deduced from the estimated daily sodium intake (see Section No.1), and the measured urinary output, that the ponies were almost certainly in negative sodium balance throughout most of the first seven days of the experiment.

From day 8 onwards, when the body reserves of sodium were being replenished, the ponies were believed to be in positive sodium balance. The marked increase in urinary sodium output exhibited by Jimmie on day 14 suggested that

over-compensation of the sodium deficit had occurred, and that the pony had still not attained a steady state of sodium balance.

This phenomenon of sodium conservation, accompanied by an initial over-compensation has also been recorded in man[179]. The possible sources of sodium drawn upon during the time when urinary sodium excretion greatly exceeded the calculated intake have already been described and discussed[23 172].

The decrease in urinary potassium output by one pony during the 12th day of the experiment was attributed chiefly to the very low volume of urine produced at that time. Metabolic acidosis in man is normally associated with hyperkalaemia and a concurrent increase in renal potassium excretion[53 179], though in sheep, intraruminal infusion of hydrochloric acid was observed to produce no change in urinary potassium excretion[98].

Although there were no significant changes in the potassium content of urine voided during ammonium chloride ingestion, a general trend was towards decreased urinary potassium loss, especially near the end of the period of ammonium chloride administration, was evident. The refusal of part of the daily hay ration, which reduced the dietary potassium intake, especially around the times that the lower urinary potassium values were noted was believed to be responsible for this trend. It was not known whether the potassium content of faecal fluid was also reduced.

The highly significant increases observed in the urinary chloride content were undoubtedly caused primarily

by the excretion of the chloride component of the acidifying salt[53]. Nicholson[89] discovered increases in the urine chloride content after the administration to ponies of both ammonium chloride and calcium chloride. Since 50g of ammonium chloride contain 935 mEq of chloride, the results listed in Table No.94, when compared with those pertaining to normal urinary chloride excretion by these ponies (see Table No. 29, Section No.1, Part 2) suggest that not all the chloride administered was excreted in the urine. The analyses of faecal fluids during this work might have revealed an increase in chloride content, and this would represent any increased secretion of chloride into the gut in addition to unabsorbed chloride. Increased urinary chloride excretion is a feature of metabolic acidosis in man even if the condition was not induced by the ingestion of a chloride salt[53]. However, the return of the urinary chloride excretion to normal quantities preceded the rise to normal of urine pH in two of the ponies, and no relationship between urine pH and chloride excretion was apparent.

During the daily ingestion of 50g of ammonium chloride, very substantial increases in urinary inorganic phosphate excretion were observed which, with the exception of one pony, returned to normal levels within twenty-four hours of the last dose. Plasma inorganic phosphate concentrations at this time were not significantly changed. It was obvious that ammonium chloride was able to evoke large increases in urinary phosphate content, and though its

buffering action could not be proved simply by estimating the inorganic phosphate content of urine it was deduced that buffering was taking place[98 106 182 183]. Other authors have described increases in urinary phosphate excretion under similar conditions in other species[173 179 183]. Christensen[183], stated that during increased phosphate excretion dogs and humans are in negative phosphate balance. Sartorius, Roemmelt and Pitts[179] observed that in man, as in these ponies, an increase in urinary inorganic phosphate occurred without any increase in phosphate level of plasma, and they deduced that the source of the phosphate was intracellular. Fulop and Brazeau[168], working with dogs, discovered that the tubular reabsorption of sodium influenced that of inorganic phosphate and hence increases in the renal excretion of sodium were accompanied by increases in renal inorganic phosphate excretion. Though their discovery might be relevant to the observations that in the ponies the renal excretion of both sodium and inorganic phosphate was increased by ammonium chloride ingestion, the times when the urinary inorganic phosphate content was abnormally large frequently did not coincide with increases in the urinary sodium content. Furthermore, renal sodium excretion returned to within normal limits before the renal inorganic phosphate excretion of two ponies decreased. Though the increased inorganic phosphate excretion exhibited by Scruffy on day 12 coincided with a 24 hour sodium excretion greater than the mean value, a concurrent decrease in pH also occurred. Hence it seemed unlikely that high

sodium excretion alone was responsible for the increased loss of inorganic phosphate.

The work of Burnell and Teubner[172] has already been described in connection with the investigations of other electrolyte changes, but at this point it is worthy of note that they found no significant change in the phosphate content of the bone samples they analysed, which were removed from acidotic dogs. Obviously this need not apply to other species, and changes in the inorganic phosphate content of bone might vary with the severity and duration of the acidosis.

However, in view of the work of Burnell and Teubner[172] and the discovery of the large quantities of inorganic phosphate normally lost in the faecal fluids of these ponies (see Section No.1, Part 3, Table No.51 and Appendix No. 3 (xiv)) it was considered possible that much of the phosphate lost during and immediately after ammonium chloride ingestion could have been ultimately derived from intestinal fluid. Even when urinary phosphate excretion was greatest it was only equal to approximately one quarter of the mean faecal loss from the untreated pony. Whether or not the ponies were in negative phosphate balance at the time of maximum urinary phosphate output was not known, since neither the dietary intake nor the faecal loss were measured.

It was concluded that though the urinary inorganic phosphate content might have been influenced by renal sodium excretion[168] it was probable that the acid load imposed upon the ponies exerted the major influence.

Although significant increases occurred in the urinary ammonium content of three out of the four ponies, these increases were not so pronounced, nor so sustained, as the increases in urinary phosphate excretion. This observation contrasts with the observations of workers who have studied the effects of metabolic acidosis in other species[98 179 184 185]. In sheep the urinary ammonium excretion after intraruminal hydrochloric acid infusion was approximately equivalent to the acid infused[98].

It has been noted in man that increases in urinary ammonium occurred within 4 hours after the ingestion of an acidifying salt[173], but since the urine analysed in the course of this work with the ponies consisted of aliquots of samples collected over longer periods it was not possible to detect whether it occurred with equal rapidity in these animals.

The administration of a constant ammonium chloride load to rats resulted in a progressive increase in renal glutaminase activity, which was closely paralleled by a similar increase in ammonium excretion[184]. Renal glutaminase in rats was the subject of a study by Longshaw and Pogson[186]. They hypothesised that the stimulation of gluconeogenisis by acidosis might reduce total glutamate - which was reputed to inhibit glutaminase activity - and thereby stimulate glutamine breakdown by glutaminase to produce ammonia.

Goldberger[53] and Rector et al.[184] proposed that the increase in urinary ammonium excretion served two functions,

namely buffering and sodium conservation.

If these discoveries are applied to the observations made upon the ponies, several important issues arise. With the exception of one pony who showed highly significant increases in urinary sodium excretion but no significant change in ammonium excretion, an inverse relationship between sodium and ammonium excretion was not apparent.

Though the ammonia buffering system apparently assumes great importance in the urinary excretion of acid in man[53] [179], in these ponies the phosphate buffer system appeared to have been the more active of the two buffers. Why ammonium excretion was not consistently elevated during ammonium chloride acidosis cannot be answered by the data obtained. The dose of the salt given was sufficient to induce acidosis. No information upon the levels of glutamine and glutaminase in equine kidneys was discovered, but the possibility that the glutamine and glutaminase contents of the kidneys of these ponies, if not those of all equine animals, were low cannot be disregarded. If inorganic phosphate is readily available for renal excretion this would likely take precedence over ammonium excretion, especially if renal glutamine and/or glutaminase levels are low.

Since no preservative was added to the urine samples the production of ammonium by urea splitting could have occurred. This would have resulted in high estimations of ammonium excretion, and so the chance that urinary ammonium was underestimated was disregarded.

50g of ammonium chloride contains 13.1g of nitrogen which in turn can be incorporated into approximately 28g of urea. This quantity of urea is roughly equivalent to twice the standard deviation of the ponies' mean daily urinary urea excretion. Hence, unless the excretion of urea from sources other than ammonium chloride exceeded the normal mean value the superimposition of urea derived from ammonium chloride would not increase the daily urinary output beyond the normal mean plus two standard deviations. Therefore the increase would not be considered significant. This could be the reason for the increases being found statistically significant only in the two ponies with the smallest standard deviations of the control means.

It was concluded that in these ponies, as in man[53], urinary urea excretion was increased during ammonium chloride loading, by the metabolism of the ammonium cation to urea.

Three factors which could have influenced the urinary urea content after ammonium chloride ingestion are failure to absorb all the ammonium chloride from the gut, retention of urea within the body, and urea splitting. It was not possible during the course of this experiment to determine whether or not all the salt was absorbed, but failure to absorb all the ammonium chloride would result in the formation of less than 28g of extra urea. Though plasma urea concentrations were increased, an increase of 50mg urea/100ml of plasma in a pony whose plasma volume was approximately 5 litres (see Section No.3) represents a total increase of about 2.5g of urea in the plasma. However, urea

diffuses freely between intra- and extracellular fluids[29], so that far more than 2.5g could be retained. If urea was retained twenty-four hours or longer after ammonium chloride ingestion the urinary urea content of a sample could be lower because of this. In view of the magnitude of daily urinary urea output, and the observation that only in one pony was the elevation in plasma urea concentrations both profound and prolonged beyond the seventh day of the experiment, it was concluded that severe urea retention was not likely to have occurred in these ponies. Hydrolysis of urea in the urine samples, with the consequent formation of ammonium has already been discussed. The complete hydrolysis of one gram of urea yields circa 33 mEq of ammonium. The possibility of this process having occurred between the times of urine being voided and the commencement of analysis cannot be disregarded as a source of a positive error in the estimated ammonium excretion, and a negative error in estimated urea excretion. The addition of a preservative to the collecting bottles in order to prevent urea splitting would have enabled more accurate determinations of the ammonium and urea content of the urine at the time it was voided to have been made, but might have interfered with other analyses.

It was unfortunate that not all urine voided by the ponies during these experiments was available for analysis, in order that a more complete investigation could have been carried out, but complete continuous collection was not considered possible on humane grounds. For obvious reasons

it was most unfortunate that all blood and urine analyses could not have been performed during the course of a single experiment. Had it been possible to have used the same dosage rates during the second experiment, the fact that the investigation had to be divided into separate experiments would not have mattered so much, but in the interests of the ponies' safety it was considered necessary to lower the rate of ammonium chloride administration.

Though Piperno et al.[170] reported no fatalities among their horses, the dose of ammonium chloride/kg body weight/24 hours which they administered, namely 360g/100lbs/24hours divided into six equal doses, was approximately three times greater than that administered to the four ponies studied in the first experiment. From the reactions of these ponies it was concluded that Piperno et al.[170] ran a very serious risk of administering a fatal overdose of ammonium chloride.

SUMMARY OF SECTION No.4

The effect of ammonium chloride ingestion upon selected constituents of the blood and urine of Shetland and Shetland-cross ponies was studied and the resulting clinical signs are described.

During ammonium chloride administration a compensated metabolic acidosis occurred, but blood pH, pCO_2 and serum bicarbonate concentrations quickly returned to normal when ammonium chloride ingestion ceased. Though significant decreases in urine pH occurred, changes from the urinary excretion of net base to net acid were considered more indicative of the renal regulation of acid/base status than urine pH. Although acid was excreted, urine pH was sometimes discovered to be greater than that of blood.

Ammonium chloride, at the doses given, induced natriuresis without a concurrent diuresis. Despite large increases in urinary sodium excretion no consistent decreases in plasma sodium concentrations were apparent, and no consistent changes in plasma potassium concentrations and urinary potassium excretion were observed.

Two ponies showed increased plasma chloride concentrations, though these increases were not sustained. High urinary chloride excretion during ammonium chloride ingestion was observed. No significant changes in plasma inorganic phosphate levels during ammonium chloride ingestion were noted, though urinary inorganic phosphate excretion was significantly increased in all the ponies studied. It is

suggested that phosphate in intestinal fluid was absorbed and excreted via the kidneys.

Plasma urea concentrations were raised in three of the four ponies, but statistically significant increases in urinary ammonium and urea excretion were not invariably observed, and when they occurred they were not sustained throughout the entire period of ammonium chloride ingestion. The possible reasons for these observations are discussed.

SECTION No.5

A STUDY OF CHANGES IN SELECTED BLOOD AND URINE CONSTITUENTS FOLLOWING SODIUM BICARBONATE ADMINISTRATION TO PONIES.

INTRODUCTION

Investigation of acid/base parameters in clinically normal untreated horses have been described and discussed in Section No.1. However, the normal ranges of pH, pCO_2 and bicarbonate concentration in equine blood do not appear to have been satisfactorily established.

Studies of metabolic alkalosis have apparently been confined to man and to animals other than the horse. No reference to changes in blood and urine constituents arising from increases in blood pH in equine species has been discovered. In other species sodium bicarbonate administration has been shown to increase blood and urine pH[53 98 172 174 175 177], and plasma bicarbonate concentration[53 174]. Decreases in plasma potassium concentrations following the oral and parenteral administration of sodium bicarbonate have been reported[53 176], and concurrent falls in plasma sodium concentration have sometimes been noted[53].

Sodium bicarbonate was administered to ponies in an attempt to determine whether the changes in blood and urine which were observed after the intake of this salt in other species also occurred in equines. Since the effect upon selected blood and urine constituents of ammonium chloride-induced metabolic acidosis has been studied (see Section No.4) it was decided to investigate the same constituents during and after sodium bicarbonate administration, in order to discover whether an increase in blood pH induced opposite changes to a decrease in blood pH.

METHODS

Four ponies were investigated during this experiment. Their general management has already been described (see Section No.1, Part 1). During each of five successive days 62.8g of sodium bicarbonate in aqueous solution was administered by stomach tube.

Blood samples were collected daily at 10.00 hours on ten consecutive days. The technique of blood sampling has been described in Section No.1, Part 1. Sodium bicarbonate was administered immediately after blood sampling on days 1 to 5 inclusive.

The ponies were confined to small stalls during days 1 to 4 whilst 24 hour urine samples were collected[94]. At other times they were housed in looseboxes. The collection of 24 hour urine samples began at 10.00 hours, as soon as the pony had received the daily dose of sodium hydrogen carbonate. For reasons already stated, following the ingestion of the final dose of sodium hydrogen carbonate on day 5, the ponies were afforded 24 hours rest in looseboxes. They returned to the stalls at 10.00 hours on day 6. 24 hour urine samples were collected on days 6, 8 and 10.

Access to drinking water ad libitum was afforded at all times. The daily 4kg hay ration was presented at 16.00 hours.

The packed cell volume percentage, blood pH, pCO_2, serum bicarbonate concentration and the concentrations in plasma of sodium, potassium, chloride, inorganic phosphate and urea were measured in every blood sample. The methods employed are described in Section No.1, Part 1. Urine

volume, pH, specific gravity and the sodium, potassium, chloride, inorganic phosphate, ammonium, urea and net base contents of the samples were determined by methods described in Section No.1, Part 2.

RESULTS

Because the number of ponies studied was small, and to avoid masking variations in individual responses to sodium bicarbonate ingestion, the results from each pony are presented singly. Each pony was used as his own control. The judgement of the significance of changes observed was based upon the mean value ± standard deviation of the parameters in each pony in the untreated state (see Section No.1, Parts 1 and 2). A value outwith the mean ± 2SD was considered statistically significant, and a value outwith the mean ± 3SD was considered highly significant.

The ponies exhibited no signs of discomfort or distress throughout the five days during which sodium bicarbonate was administered. No changes in the rate or depth of respiration were observed, and all ponies consumed the whole of each daily hay ration.

KEY TO SYMBOLS USED IN THE TABLES

* Denotes a significant increase ($p < 0.05$)

\+ Denotes a highly significant increase ($p < 0.01$)

/ Denotes a significant decrease ($p < 0.05$)

x Denotes a highly significant decrease ($p < 0.01$)

TABLE No.98

THE EFFECT OF SODIUM BICARBONATE UPON BLOOD pH

		$NaHCO_3$ Administration	Jimmie	Billie	Ben	MacGowan
Before	$NaHCO_3$	none	7.390	7.420	7.400	7.380
Day	1	62.8g	7.400	7.410	7.450	7.450+
"	2	62.8g	7.425	7.435	7.400	7.400
"	3	62.8g	7.435	7.440	7.450	7.410
"	4	62.8g	7.430	7.440	7.420	7.410
"	5	62.8g	7.380	7.410	7.370	7.400
"	6	none	7.390	7.420	7.450	7.400
"	7	none	7.410	7.420	7.400	7.380
"	8	none	7.410	7.410	7.390	7.400
"	9	none	7.390	7.400	7.450	7.410

TABLE No.99

THE EFFECT OF SODIUM BICARBONATE UPON BLOOD pCO_2 (mmHg)

		$NaHCO_3$ Administration	Jimmie	Billie	Ben	MacGowan
Before	$NaHCO_3$	none	45.5	42.5	44.0	47.5
Day	1	62.8g	45.0	46.5	41.5	43.5
"	2	62.8g	45.0	41.0	45.5	46.0
"	3	62.8g	48.0	41.0	39.5	44.0
"	4	62.8g	48.0	42.5	41.5	46.5
"	5	62.8g	52.0*	42.0	47.0	47.0
"	6	none	50.5	43.0	39.5	46.0
"	7	none	47.5	41.0	42.5	45.5
"	8	none	48.5	44.0	48.0	47.5
"	9	none	46.0	40.0	42.5	46.0

TABLE No.100

THE EFFECT OF SODIUM BICARBONATE UPON SERUM BICARBONATE CONCENTRATION (mEq/l)

		$NaHCO_3$ Administration	Jimmie	Billie	Ben	MacGowan
Before $NaHCO_3$		none	26.3	27.0	26.3	27.0
Day	1	62.8g	26.0	26.9	27.8	29.2
"	2	62.8g	28.3	29.1	27.2	27.8
"	3	62.8g	34.1+	27.1	26.8	27.1
"	4	62.8g	29.9	27.9	25.7	28.0
"	5	62.8g	29.7	25.8	26.5	28.1
"	6	none	29.6	26.9	26.4	27.6
"	7	none	29.4	25.9	25.4	25.9
"	8	none	27.7	27.1	28.1	28.5
"	9	none	27.9	24.2x	29.0	27.6

Few significant changes in blood pH, pCO_2 and serum bicarbonate concentrations were observed, and during the period of sodium bicarbonate ingestion these changes were confined to two ponies. Alkalosis occurred in one pony (MacGowan), on a single occasion only. Though Billie exhibited an abnormally low serum bicarbonate concentration and a low pCO_2 on day 9, the blood pH remained within normal limits.

TABLE No.101

THE EFFECT OF SODIUM BICARBONATE UPON PACKED CELL VOLUME PERCENTAGE

		$NaHCO_3$ Administration	Jimmie	Billie	Ben	MacGowan
Before	$NaHCO_3$	none	29.0	32.5	38.5	31.5
Day	1	62.8g	31.0	31.0	39.5	31.0
"	2	62.8g	30.5	32.5	37.5	29.5
"	3	62.8g	31.5	31.0	42.5	30.5
"	4	62.8g	27.5	30.5	38.0	30.5
"	5	62.8g	29.5	30.5	37.5	33.0
"	6	none	29.5	31.5	39.0	32.5
"	7	none	28.5	30.0	36.5	30.5
"	8	none	28.5	28.0	37.0	31.5
"	9	none	28.5	30.0	37.0	29.0

No significant changes in the packed cell volume percentage of any pony occurred during the experiment.

TABLE No.102

THE EFFECT OF SODIUM BICARBONATE UPON PLASMA SODIUM CONCENTRATION (mEq/l)

		$NaHCO_3$ Administration	Jimmie	Billie	Ben	MacGowan
Before	$NaHCO_3$	none	130	136	135	135
Day	1	62.8g	130	134	135	138
"	2	62.8g	140	139	135	136
"	3	62.8g	130	139	133	135
"	4	62.8g	128	138	133	139
"	5	62.8g	128	138	131	136
"	6	none	130	135	128	135
"	7	none	135	134	135	135
"	8	none	133	134	133	135
"	9	none	135	136	130	140

The plasma sodium concentrations of all the ponies remained within normal limits at all times.

TABLE No.103

THE EFFECT OF SODIUM BICARBONATE UPON PLASMA POTASSIUM CONCENTRATION (mEq/l)

		$NaHCO_3$ Administration	Jimmie	Billie	Ben	MacGowan
Before	$NaHCO_3$	none	5.40	4.20	4.20	3.20[x]
Day	1	62.8g	4.50	4.00	3.90	3.40
"	2	62.8g	5.30	4.70[*]	3.90	3.80
"	3	62.8g	5.00	3.70	4.40	3.80
"	4	62.8g	5.00	3.85	3.75	4.30[*]
"	5	62.8g	5.10	4.00	4.10	3.90
"	6	none	4.65	3.60	3.70	3.90
"	7	none	4.25	3.90	3.75	3.85
"	8	none	5.10	3.70	3.60	3.85
"	9	none	5.00	3.90	3.90	4.10

Though the plasma potassium concentrations of two ponies changed significantly during the period of daily sodium bicarbonate ingestion the changes were transient. An increase above normal in Billie's plasma potassium concentration occurred on day 2. MacGowan commenced the experiment with an abnormally low plasma potassium level, and a significant increase was observed on day 4.

TABLE No.104

THE EFFECT OF SODIUM BICARBONATE UPON PLASMA CHLORIDE CONCENTRATION (mEq/l)

		$NaHCO_3$ Administration	Jimmie	Billie	Ben	MacGowan
Before	$NaHCO_3$	none	96	103	101	100
Day	1	62.8g	100	103	99	97
"	2	62.8g	99	104	102	99
"	3	62.8g	97	104	101	100
"	4	62.8g	98	102	100	99
"	5	62.8g	99	103	102	102
"	6	none	97	104	101	101
"	7	none	99	106*	103	101
"	8	none	101	102	99	101
"	9	none	98	101	100	100

A single singificant increase in plasma chloride concentration occurred in Billie, two days after sodium bicarbonate ingestion ceased. No other changes in this plasma constituent were observed.

TABLE No.105

THE EFFECT OF SODIUM BICARBONATE UPON PLASMA INORGANIC PHOSPHATE CONCENTRATION (mgP/100ml)

		$NaHCO_3$ Administration	Jimmie	Billie	Ben	MacGowan
Before	$NaHCO_3$	none	1.45/	2.20	3.71	2.85
Day	1	62.8g	1.63/	3.60	4.25	1.75
"	2	62.8g	2.12	3.86	3.08	2.38
"	3	62.8g	1.32x	5.59+	3.31	2.56
"	4	62.8g	2.28	5.97+	2.36/	2.52
"	5	62.8g	1.80/	6.71+	2.05/	1.90
"	6	none	1.18x	5.89+	2.89	2.66
"	7	none	1.91/	4.94+	2.87	2.67
"	8	none	1.10x	1.86/	3.17	1.84
"	9	none	1.97/	2.98	3.58	1.82

Three ponies exhibited pronounced changes in plasma inorganic phosphate concentrations, but the patterns of change were inconsistent. Although one pony (Jimmie) showed highly significant decreases in his plasma inorganic phosphate concentrations he commenced the experiment with an abnormally low level, so obviously this needs to be borne in mind when interpreting the effect of the salt upon this plasma constituent. Ben exhibited a significantly lowered plasma inorganic phosphate concentration on days 4 and 5.

Billie commenced the experiment with a normal plasma inorganic phosphate concentration which remained within normal limits for two days. During days 3, 4, 5, 6 and 7 the concentration was abnormally high. On day 8 the inorganic phosphate concentration was significantly decreased and only on the last day of the experiment did the concentration return to normal.

TABLE No.106

THE EFFECT OF SODIUM BICARBONATE UPON PLASMA UREA CONCENTRATION (mg urea/100 ml)

		$NaHCO_3$ Administration	Jimmie	Billie	Ben	MacGowan
Before $NaHCO_3$		none	53.5	33.8	26.0	23.9
Day	1	62.8g	49.8	31.4	26.4	15.6$^{\checkmark}$
"	2	62.8g	51.6	29.4	16.9	21.0
"	3	62.8g	36.5	36.2	17.9	20.4
"	4	62.8g	39.4	33.1	14.7$^{\checkmark}$	17.1
"	5	62.8g	38.1	30.7	11.2^{x}	21.0
"	6	none	51.2	24.0	14.2$^{\checkmark}$	22.4
"	7	none	48.2	40.0	15.8$^{\checkmark}$	31.3
"	8	none	53.3	25.6	17.8	22.0
"	9	none	41.4	25.9	18.7	21.3

Plasma urea concentrations were significantly reduced in two ponies only. In one of these two, (Ben), abnormally low levels were observed at times during and after sodium bicarbonate ingestion. An abnormally low plasma urea concentration in the second pony (MacGowan) was observed on one occasion during sodium bicarbonate ingestion.

TABLE No.107

THE EFFECT OF SODIUM BICARBONATE UPON URINE VOLUME/24 HOURS

(ml)

		$NaHCO_3$ Administration	Jimmie	Billie	Ben	MacGowan
Day	1	62.8g	5110	8790+	3620	4640
"	2	62.8g	5210	4990	4510	4410
"	3	62.8g	5850*	3980	4260	6040
"	4	62.8g	6800+	7270*	3160	6390
"	5	62.8g	——— Urine not collected ———			
"	6	none	4390	5100	2580	4680
"	8	none	3730	1850	2890	2660
"	10	none	3220	3180	3420	5530

TABLE No.108

THE EFFECT OF SODIUM BICARBONATE UPON URINE SPECIFIC GRAVITY

		$NaHCO_3$ Administration	Jimmie	Billie	Ben	MacGowan
Day	1	62.8g	1.027	1.022	1.031	1.032
"	2	62.8g	1.031	1.027	1.032	1.032
"	3	62.8g	1.027	1.035	1.030	1.021
"	4	62.8g	1.026	1.024	1.034	1.024
"	5	62.8g	——— Urine not collected ———			
"	6	none	1.032	1.034	1.045	1.028
"	8	none	1.024	1.037	1.028	1.041
"	10	none	1.032	1.026	1.033	1.017

Increases beyond normal limits in the volumes of urine voided/24 hours were observed intermittently in two ponies only. No decreases in urine specific gravity occurred even when urine volume/24 hours was increased.

TABLE No.109

THE EFFECT OF SODIUM BICARBONATE UPON URINE pH

		$NaHCO_3$ Administration	Jimmie	Billie	Ben	MacGowan
Day	1	62.8g	9.05	9.00	8.85	8.80
"	2	62.8g	9.20	9.10	9.05	8.85
"	3	62.8g	9.15	8.90	9.05	9.00
"	4	62.8g	9.10	8.90	9.10	8.90
"	5	62.8g	——— Urine not collected ———			
"	6	none	9.10	8.40	8.75	8.75
"	6	none	9.15	8.80	9.10	8.75
"	10	none	9.10	8.85	8.95	8.55

TABLE No.110

THE EFFECT OF SODIUM BICARBONATE UPON THE ACID/BASE CONTENT OF URINE (mEq/24 hours)

		$NaHCO_3$ Administration	Jimmie	Billie	Ben	MacGowan
Day	1	62.8g	953^+	1642^+	920^*	730
"	2	62.8g	1413^+	956^+	1133^+	1330^*
"	3	62.8g	1128^+	843^+	1006^+	904
"	4	62.8g	974^+	921^+	766	1121
"	5	62.8g	——— Urine not collected ———			
"	6	none	823^+	713^+	411	387
"	8	none	433	76	376	358
"	10	none	506	153	483	124

N B. All results listed in Table No. 110 are mEq of Net Base.

No statistically significant changes in urine pH occurred during or after sodium bicarbonate ingestion. Though the pH of the urine voided by Jimmie during days 6, 8, and 10 remained high, in all other ponies the mean pH of the urine collected after the cessation of sodium bicarbonate ingestion was lower than the urine pH values observed during days 1 to 4.

Net base excretion was increased in all ponies, though in Ben the increase occurred only during the first three days of the experiment, and MacGowan only once exhibited a significant increase in urinary base excretion. When hydrochloric acid was added to the urine at the commencement of net acid/base determinations a very pronounced effervescence of the urine samples was observed. This was considered to indicate a high bicarbonate content[95].

TABLE No.111

THE EFFECT OF SODIUM BICARBONATE UPON THE SODIUM CONTENT OF URINE (mEq/24 hours)

		$NaHCO_3$ Administration	Jimmie	Billie	Ben	MacGowan
Day	1	62.8g	626+	659+	705+	278
"	2	62.8g	581+	649+	870+	457*
"	3	62.8g	623+	378+	780+	544+
"	4	62.8g	782+	836+	525+	671+
"	5	62.8g	——— Urine not collected ———			
"	6	none	241	159	263	240
"	8	none	126	42	206	136
"	10	none	37	108	278	76

The sodium content of urine samples voided by all ponies was very markedly increased during sodium bicarbonate ingestion. However, sodium excretion/24 hours only surpassed the sodium ingested in the form of sodium bicarbonate in four instances. 24 hour urinary sodium excretion returned to within the established normal limits in all ponies immediately the administration of sodium bicarbonate ceased. No significant fluctuations in this urine constituent were observed on days 6, 8, and 10.

TABLE No.112

THE EFFECT OF SODIUM BICARBONATE UPON THE POTASSIUM CONTENT OF URINE (mEq/24 hours)

		$NaHCO_3$ Administration	Jimmie	Billie	Ben	MacGowan
Day	1	62.8g	1099	1785*	1086	1346
"	2	62.8g	1407	973	1195	1499
"	3	62.8g	1404	1274	873	966
"	4	62.8g	1326	1309	727	1182
"	5	62.8g	——— Urine not collected ———			
"	6	none	1449	1683	1135	1404
"	8	none	895	555	780	1144
"	10	none	1191	716	1094	1051

Other than the usually large quantity of potassium excreted by Billie on day 1, no significant changes in urinary potassium excretion occurred at any time.

TABLE No.113

THE EFFECT OF SODIUM BICARBONATE UPON THE CHLORIDE CONTENT OF URINE (mEq/24 hours)

		$NaHCO_3$ Administration	Jimmie	Billie	Ben	MacGowan
Day	1	62.8g	485	721	456	701
"	2	62.8g	458	469	451	528
"	3	62.8g	696	525	537	550
"	4	62.8g	1027+	785	351	684
"	5	62.8g	——— Urine not collected ———			
"	6	none	619	864	769	987
"	8	none	450	394	419	787
"	10	none	528	531	728	935

Jimmie's urinary chloride excretion was exceptionally high on day 4. Otherwise no changes outwith normal limits occurred in any pony.

TABLE No.114

THE EFFECT OF SODIUM BICARBONATE UPON THE INORGANIC PHOSPHATE CONTENT OF URINE (mgP/24 hours)

		$NaHCO_3$ Administration	Jimmie	Billie	Ben	MacGowan
Day	1	62.8g	24.4	61.5	658.1	83.3
"	2	62.8g	165.6	331.2+	958.4+	97.0
"	3	62.8g	203.2	214.4+	1172.8+	69.2
"	4	62.8g	95.2	348.1+	639.0	49.7
"	5	62.8g	——— Urine not collected ———			
"	6	none	61.7	378.2+	451.0	102.5
"	8	none	61.6	80.0	1014.7+	88.6
"	10	none	6.3	61.1	988.4+	43.5

Highly significant increases in urinary inorganic phosphate excretion both during and after sodium bicarbonate

ingestion were observed in two ponies. In the case of one pony (Billie) urinary inorganic phosphate excretion became normal on day 8, and remained normal.

The urinary inorganic phosphate content of the second pony (Ben) was elevated on days 2 and 3, and then fell to normal until days 8 and 10, when highly significant increases occurred again.

TABLE No.115

THE EFFECT OF SODIUM BICARBONATE UPON THE AMMONIUM CONTENT OF URINE (mEq/24 hours)

		$NaHCO_3$ Administration	Jimmie	Billie	Ben	MacGowan
Day	1	62.8g	545*	454	98	429
"	2	62.8g	859+	232	370	333
"	3	62.8g	738+	284	278	718
"	4	62.8g	782+	316	319	472
"	5	62.8g	———	Urine not collected		———
"	6	none	503*	188	203	390
"	8	none	420	322	275	317
"	10	none	304	286	304	376

Despite the pH of the urine voided during the period of sodium bicarbonate ingestion being high, and despite large concurrent increases in urinary base excretion, one pony (Jimmie) excreted urine with an abnormally high ammonium content throughout the whole of this time.

TABLE No.116

THE EFFECT OF SODIUM BICARBONATE UPON THE UREA CONTENT OF URINE (g urea/24 hours)

	$NaHCO_3$ Administration	Jimmie	Billie	Ben	MacGowan
Day 1	62.8g	24.7	41.7	27.5	35.7
" 2	62.8g	17.0	14.4	34.4	33.3
" 3	62.8g	20.8	37.5	28.4	20.2
" 4	62.8g	13.5	44.8	23.8	32.2
" 5	62.8g	——— Urine not collected ———			
" 6	none	14.9	68.3*	18.4	31.3
" 8	none	18.2	30.8	20.6	33.4
" 10	none	17.5	20.9	25.1	28.1

Only one pony (Billie) showed a change in urinary urea excretion. On day 6 a significant increase in this urine constituent was observed.

DISCUSSION

It is known that the horse is unable to vomit[187 188], and it is assumed that eructation is also impossible due to the cardiac sphincter preventing gastric gases from entering the oesophagus. Hence it was anticipated that discomfort might have resulted from the dilatation of the stomach by carbon dioxide produced when the sodium bicarbonate solution entered the acid medium of the equine stomach[23 103]. Since neither discomfort nor eructation was observed in any of the ponies, it was probable that because sodium bicarbonate was administered as a solution it passed rapidly round the lesser curvature of the stomach and then entered the far less acidic medium of the proximal small intestine[23 189]. If this occurred then little carbon dioxide would have been produced in the stomach, and any gas produced in the small intestine would likely have been produced sufficiently slowly for it to dissolve and/or dissipate without causing discomfort to the animal.

A dose of 62.8g of sodium bicarbonate was selected because it was equivalent to 40g of ammonium chloride (see Section No.4). A direct comparison of the effects of the two substances upon blood pH, pCO_2, serum bicarbonate concentration, and the volume, pH and renal net acid/base secretion of urine was therefore possible, as both salts were administered over five consecutive days.

Because only one pony showed a significant increase in blood pH, and because this increase occurred only on day 1 it was concluded that at the dose rate adopted sodium

bicarbonate was ineffective in producing a metabolic alkalosis in these ponies. It was evident that sodium bicarbonate did not induce such profound changes in blood pH as an equivalent dose of ammonium chloride.

From Tables 98, 99, and 100, it was obvious that with the exception of MacGowan on day 1 appropriate fluctuations in pCO_2 and serum bicarbonate concentrations had maintained the blood pH within normal limits. Because the magnitude of these fluctuations was small it was concluded that the dose of sodium bicarbonate had presented only a small challenge to the blood buffers.

Since the mean blood pH values of the ponies during days 1 to 5 were greater than the mean values during days 6 to 9 it is probable that higher doses of sodium bicarbonate would have induced metabolic alkalosis. However, the administration of a large dose would have defeated one object of the experiment, namely the comparison of the blood pH changes effected by equivalent doses of ammonium chloride and sodium bicarbonate.

From publications reviewed[174 177], it appeared that the parenteral infusion of an alkalising agent would have caused a more profound change in acid/base status than the administration per os of an equivalent amount of the substance.

For future acid/base studies, the parenteral administration of acidifying and alkalising agents would be worthwhile, and would eliminate any variations caused by differences in intestinal absorption. Evidence for a high percentage of the administered sodium bicarbonate being

absorbed was provided by the large increases in urinary net base excretion at this time, but due to fluctuations in the net base excretion of the untreated ponies (see Section No.1, Part 2, and Appendices 2 [(iv) and (xii)]) it was impossible to calculate the exact proportion of sodium bicarbonate which was absorbed.

The lack of change in the packed cell volume percentage was anticipated. It has already been shown that the packed cell volume percentage is a very poor indicator of changes in plasma volume (see Section No.3). Since the ponies incurred no consistent diuresis, and because drinking water was available at all times during the experiment, no significant changes in the plasma volumes were expected.

The lack of marked changes in the plasma sodium concentrations was somewhat surprising. 62.8g of sodium hydrogen carbonate contains 748 mEq of sodium, a quantity approximately equivalent to, or slightly greater than, the total sodium present in the plasma of each pony (see Section No.3, Table No.61), and yet the ponies were able to absorb and excrete a large quantity of sodium and so regulate their plasma sodium concentrations that no significant changes occurred.

The increases in urinary sodium excretion which occurred during sodium hydrogen carbonate ingestion were considered additional proof that a large proportion of the salt was absorbed.

The slight trend towards increased plasma sodium levels

could indicate a mild degree of transient sodium retention in the extracellular fluid, though the proportion of the administered sodium which could be accounted for in this way would be small. In a pony with a thiocyanate space volume of 40 litres an increase in plasma sodium concentration of 4 mEq/l would, assuming that plasma sodium changes reflected an overall change throughout the extracellular fluid, indicate an increase in extracellular fluid sodium of 160 mEq, which is little more than 20% of the sodium content of 62.8g of sodium hydrogen carbonate.

Two other possible sites of sodium retention might have been involved in maintaining normal plasma sodium levels during the absorption of large quantities of this element. It is possible that the rate of sodium absorption from the gut was regulated such that it closely approximated with the renal excretory rate. Furthermore, Burnell and Teubner's[172] observation that the sodium content of bone increases during metabolic alkalosis in dogs might be relevant. Although it was concluded, with the exception of one pony on one occasion, that metabolic alkalosis did not occur, blood pH values were generally higher during days 1 to 5 than during days 6 to 9. Burnell and Teubner[172] did not state whether the deposition of sodium in bone during alkalosis occurred only above a specific blood pH "threshold" or whether changes in the extent of sodium deposition were related to blood pH changes both within and without normal pH ranges. If the latter occurred then maybe even the small blood pH changes observed in the ponies could have resulted in some skeletal sodium retention.

Despite the intermittent changes in plasma potassium concentrations exhibited by two of the ponies it was concluded that sodium bicarbonate at this dose rate caused no distinct consistent changes in the plasma potassium levels. It has been reported in other species that alkalosis causes hypokalaemia[53 176 177 182], due to the entry of potassium into cells. The quantitative inverse pH/plasma potassium concentration relationship which has been observed in other species[177 182 190] was never evident during the course of this work. The significant changes in plasma potassium concentrations coincided with normal blood pH values, and hence it was concluded that they occurred randomly. It was not known why MacGowan exhibited an abnormally low plasma potassium concentration at the beginning of the experiment. Had the ponies been alkalotic more profound changes might have arisen, and from these a distinct trend may have been evident.

Whereas sodium bicarbonate did not cause any significant decreases in plasma chloride concentrations, it was interesting to note that the administration of an equivalent quantity of ammonium chloride to these ponies caused significant decreases in their serum bicarbonate concentrations. The pony who showed the lowest plasma chloride levels simultaneously exhibited the greatest increase in serum bicarbonate concentration, but only in this pony (Jimmie) was there any indication of reciprocal fluctuations in bicarbonate and chloride concentrations[178]. It is probable that higher doses of sodium bicarbonate, or the

parenteral infusion of a sodium bicarbonate solution might induce a metabolic alkalosis accompanied by large increases in serum bicarbonate, and concurrent reciprocal changes in plasma chloride concentrations might then arise.

The changes in the plasma inorganic phosphate concentrations of the ponies both during and after sodium bicarbonate ingestion are difficult to explain. Since blood acid/base disturbances were minimal, the changes could not readily be attributed to alkalosis. Furthermore no two ponies exhibited a similar pattern of change.

It was concluded that the plasma inorganic phosphate concentration of MacGowan was unaffected. Since Jimmie began the experiment with an abnormally low plasma inorganic phosphate concentration, the low levels which persisted on all but two days of the experiment could hardly be attributed to sodium bicarbonate ingestion. Though the decreases in the plasma inorganic phosphate concentrations of Ben were significant, they were neither large nor sustained, but they followed significant increases in urinary inorganic phosphate excretion which occurred on days 2 and 3. However, since total inorganic phosphate intake and output was not measured it was not known whether the decreases in the plasma inorganic phosphate concentration were indicative of depletion or a delay in the mobilisation of the phosphate resources of the pony.

No publication reviewed suggest the cause of the increases during days 3 to 7 in the plasma inorganic phosphate concentrations of Billie. If the changes in plasma

inorganic phosphate concentration were secondary to changes in the urinary excretion of inorganic phosphate it can only be assumed that this pony in some way over-reacted to the increased urinary loss which tended to deplete the plasma inorganic phosphate content. Why two ponies who both exhibited increased urinary phosphate losses showed contrasting fluctuations in their plasma inorganic phosphate concentrations is unknown. The abnormally low value observed in Billie on day 8 probably indicated difficulty in regulation, before the plasma inorganic phosphate concentration returned to normal on day 9. Although increases in the urinary inorganic phosphate content of this pony also occurred during ammonium chloride administration (see Section No.4), no concurrent changes in the plasma inorganic phosphate levels took place then.

The source of the additional inorganic phosphate in the blood was not known; but two possibilities are the inorganic phosphate of the gut fluids[23] and the cells of the body[53]. Inorganic phosphate might have been absorbed from the gut when increased urinary inorganic phosphate loss occurred. Alternatively, or in addition, since most phosphate in the soft tissues of the body is intracellular[53], loss of inorganic phosphate from the cells might have occurred.

No reference to the effect of alkalising salts upon urea metabolism in equines was discovered. The reasons for the falls below normal of the plasma urea levels of two ponies are not understood. Although MacGowan only once

exhibited a low plasma urea concentration, the fact that in Ben significantly reduced levels occurred over days 4 to 7 inclusive suggested that the decreases were not due to chance. All ponies consumed all the daily hay ration, and the times of blood sampling in relation to feeding were constant, hence the changes in blood urea levels were unlikely to be caused by changes in feeding patterns (see Section No.2).

No correlation between the low plasma urea concentrations and blood pH was evident. In the case of MacGowan the low plasma urea level coincided with the rise above normal of blood pH. However, the blood pH of Ben over days 4 to 7 ranged from 7.370 to 7.450, and these values fell within the normal range of blood pH for this pony. Neither of the ponies in whom low plasma urea concentrations were detected showed any significant change in the daily urinary ammonium or urea excretion.

The fact that the low plasma urea levels observed in Ben occurred both during and after sodium bicarbonate ingestion suggests that sodium bicarbonate alone did not influence the plasma urea concentration.

Since increases in the 24 hour urine volume were evident in only four individual samples voided during the period of sodium bicarbonate ingestion it was concluded that sodium bicarbonate at this dose rate caused no pronounced diuresis by these ponies. Similarly, an equivalent dose of ammonium chloride also caused increases in the volume of urine voided/24 hours on four occasions. It is

worthy of note that during the ingestion of sodium bicarbonate and during the ingestion of ammonium chloride the ponies were able to excrete unusually large quantities of sodium without concurrent increases in urine volume. This observation will be discussed later.

Evidently the intermittent increases in the urine volumes voided by two of the ponies were insufficient to bring about significant decreases in the urine specific gravity[89].

The lack of significant increases in urine pH during sodium bicarbonate ingestion contrasts markedly with the significant decreases observed when ammonium chloride was administered. If all the bicarbonate ingested daily in the form of 62.8g of sodium bicarbonate was excreted in the urine within 24 hours this would represent an additional 748 mEq of bicarbonate. 748 mEq of bicarbonate in 5430 ml of urine (the mean volume voided over days 1 to 4) represents a bicarbonate concentration of 138 mEq/litre. It was discovered that if sodium bicarbonate was added to urine samples of pH range 7.80 to 8.30 *in vitro* at the rate of 10g of sodium bicarbonate/litre of urine, or 120 mEq of bicarbonate/litre, maximum increases in urine pH of approximately 0.2 unit occurred[191]. Hence it was not anticipated that the ingestion of 62.8g of sodium bicarbonate would cause significant increases in urine pH. Furthermore, the action of any urinary buffers which were active in the 8.4 to 9.2 pH range might tend to minimise pH changes.

The increase in urinary base excretion during sodium

bicarbonate ingestion was anticipated, and was probably due to increased renal bicarbonate excretion, although the increase which was observed in renal inorganic phosphate excretion would also contribute, since the proportion of inorganic phosphate present as the monohydrogen anion increases with urine pH increases[53 105 106].

The trend towards increased urinary base excretion by MacGowan during sodium bicarbonate ingestion was obvious, even though the increase was significant only on day 2. The quantity of base excreted by this pony was similar to that excreted by the other ponies, and the lack of statistical significance was attributed to the fact that the standard deviation of the mean daily net base excretion of this pony was greater than that of the others (see Table No.26, Section No.1, Part 2). The day-to-day fluctuations in the net base excretion of all ponies during days 1 to 4 were probably due partly to variations in the times of micturition during the 24 hour urine collections.

Although base might have been lost in faecal fluids, it was demonstrated in the untreated ponies that net acid/base loss via this route was so small that for most purposes this source of loss can be ignored (see Section No.1, Part 3).

It was expected that ammonium chloride and sodium bicarbonate would exert opposite effects upon net acid/base excretion, and this was verified. However, the estimated porportion of the base given in the form of sodium bicarbonate which could be accounted for by increased urinary net base excretion was greater than the estimated proportion

of the acid load which could be accounted for by decreased net base/increased net acid excretion in urine. It is suggested that whereas respiratory compensation is able to reduce the quality of carbonic acid present in the body by increasing the excretion of carbon dioxide via the lungs, the respiratory mechanism involved in minimising blood pH changes following sodium bicarbonate ingestion causes increases in pCO_2 in an effort to neutralise the increase in base until the latter is excreted via the kidneys[53]. It was concluded that the net base content of the urine was a better indicator of the acid/base status of the animal, and its renal response to changes in acid/base status, than urine pH.

It was evident both from this work and the work described in Section No.4 that these ponies could excrete abnormally large quantities of sodium via the kidneys without consistent concurrent increases in urine volume, and it is also evident that natriuresis without diuresis can take place regardless of whether the source of sodium is endogenous or exogenous.

Some fluctuations in daily sodium excretion would undoubtedly have been caused by variations in the times of micturition. Since some urinary sodium would have been derived from hay[97], when the mean daily excretion of sodium did not exceed 748 mEq (the quantity contained in 62.8g of sodium bicarbonate) it was obvious that not all the sodium ingested was excreted in urine. The failure to trace all the sodium ingested might have been caused by:-

a) retention

b) excretion via another route

c) a + b.

It was stated earlier that if temporary retention occurred it would most likely only account for little of the excess sodium ingested. It is probable that excretion via another route occurred, and the most obvious route is faecal excretion. However, without measuring the faecal sodium content it is impossible to prove whether this would account for all the untraced sodium, or whether some was retained and/or excreted via a non-renal route. Had increases in the sodium content of the faeces of these ponies been detected during days 1 to 4, the increases may have arisen due to the excretion of excess sodium into the gut lumen, failure to absorb all the sodium ingested in the form of sodium bicarbonate, or both.

Though one pony (Billie) excreted an unusually large quantity of potassium on day 1, this coincided with an increased urine volume, and so urinary potassium concentration was within normal limits. The reason for this single increase was unknown. It appeared from the results obtained that sodium and potassium did not compete for urinary excretion, since despite large increases in the urinary sodium content, potassium excretion was unaffected.

The unusually large quantity of chloride excreted by Jimmie on day 4 coincided with an increased volume of urine voided. It was not associated with decreases in pH and net base excretion. It was concluded that potassium and chloride excretion in the urine of these ponies was unaffected by sodium bicarbonate ingestion at this dose rate.

The only pony who failed to show any change in urinary inorganic phosphate excretion during and after sodium bicarbonate ingestion was the pony whose plasma inorganic phosphate concentration remained within normal limits. It was concluded that sodium bicarbonate induced increases in urinary inorganic phosphate in the other three ponies, even though the increases were not statistically significant in Jimmie, and despite an increased inorganic phosphate content in the urine voided by Ben on days 9 and 10 as well as during days 2 and 3. Because there was no apparent correlation between the pH of the urine and the inorganic phosphate content it was concluded that the increases observed were not mediated through the effect of sodium bicarbonate upon urine pH. Neither was there a close correlation between net acid/base excretion and inorganic phosphate excretion.

It is probable that Fulop and Brazeau's[168] observation that increases in urinary sodium excretion by dogs induced concurrent increases in urinary inorganic phosphate loss has some relevance to this work. These workers were not able to ascertain whether this phenomenon was mediated electrostatically, or whether changes in the tubular reabsorption of sodium, which in turn caused changes in water reabsorption, altered the luminal concentration of phosphate and hence its gradient for reabsorption. However, the discovery of Fulop and Brazeau[168], when applied to these ponies raised several questions which were not answered from results obtained from this experiment.

Unlike the dogs observed by Fulop and Brazeau[168], changes in sodium excretion by these ponies did not appear to effect marked consistent changes in water reabsorption. Furthermore, the inorganic phosphate content of the urine did not appear to relate in any way to the 24 hour volume. It was not known why all ponies did not incur increased urinary inorganic phosphate loss despite exhibiting similar increases in urinary sodium loss. In addition, even in Billie and Ben where the magnitude of the increases in urinary inorganic phosphate was greatest, the days upon which the excretion of inorganic phosphate was maximal did not coincide with the days of maximum sodium output. Furthermore, although urinary sodium loss returned to within normal limits very promptly after sodium bicarbonate ingestion ceased, the same was not true of inorganic phosphate excretion.

It was observed during and after ammonium chloride administration that the urinary content of both sodium and inorganic phosphate was increased (see Section No.4). Though the inorganic phosphate content of the urine samples collected during ammonium chloride administration was generally greater than that during sodium bicarbonate ingestion, the sodium content of the urine voided during ammonium chloride administration was less than that during sodium bicarbonate ingestion. This is considered to be further evidence that no simple relationship exists between the urinary excretion and the two ions. Hence it was concluded that if sodium excretion influenced the excretion of inorganic phosphate, the relationship between the two ions

was complex, and may have been influenced by other factors.

Possible sources of the excess urinary inorganic phosphate[23 53] have already been discussed previously in this section, and in Section No.4. It was considered unlikely that urinary inorganic phosphate buffering assumed much importance in the urine voided during sodium bicarbonate ingestion.

The reason for Jimmie excreting very large quantities of urinary ammonium during days 1 to 6 is unknown. The return to within normal limits of urinary ammonium excretion coincided with the return to normal of net base excretion. It is well known that ammonium buffering occurs in acidosis[53 105 106], and this was demonstrated in Section No.4, but no reference to enhanced urinary ammonium excretion during the administration of an alkalising agent was discovered.

Whilst urea splitting in vitro could not be excluded as a possible cause of increased urinary ammonium content it was considered highly unlikely that this alone was responsible for such a large urinary ammonium content which was confined to one pony during the period of sodium bicarbonate ingestion. It was concluded that for reasons unknown this pony showed a grossly abnormal response to sodium bicarbonate ingestion, and that the remaining three ponies reacted in the manner predicted by exhibiting no change in urinary ammonium excretion.

Because the only significant increase observed in urinary urea excretion occurred after sodium bicarbonate

ingestion ceased, it was concluded that sodium bicarbonate ingestion by these ponies at the dose rate employed caused no change in daily urinary urea excretion.

SUMMARY OF SECTION No.5

The effect of orally administered sodium bicarbonate upon selected constituents of the blood and urine of Shetland and Shetland-cross ponies was studied.

Few significant changes in blood pH, pCO_2 and the serum bicarbonate concentrations were observed. Although urine pH values remained within normal limits, urinary base excretion was significantly increased. It was concluded that the net acid/base excretion was a more accurate indication of the acid/base status of the ponies, and their renal response to acid/base disturbances, than urine pH.

Despite the daily ingestion of an extra 748 mEq of sodium the plasma sodium concentrations remained within normal limits, though all the ponies exhibited highly significant increases in renal sodium excretion, with few concurrent increases in the volume of urine voided. In view of the large increases in the urinary sodium content it was concluded that sodium bicarbonate at the dose rate employed induced natriuresis without a concurrent diuresis. Furthermore, since both sodium bicarbonate and ammonium chloride induced natriuresis without diuresis, this phenomenon in these ponies occurred regardless of whether the source of the sodium excreted was endogenous of exogenous.

Changes in plasma potassium concentration and the urinary potassium content were few and transient. It was therefore concluded that sodium and potassium did not compete for renal excretion. Fluctuations in blood pH and plasma potassium concentrations appeared unrelated.

A trend towards reciprocal variations in plasma chloride and serum bicarbonate concentrations was observed in only one pony, and at no time during sodium bicarbonate ingestion did the plasma chloride concentration of any pony deviate from normal. Daily urinary chloride excretion was unchanged.

Marked differences between ponies in the pattern of changes of the plasma inorganic phosphate concentration were observed, and the possible causes of these changes are discussed. Increases in the urinary inorganic phosphate excretion of three ponies occurred. One pony failed to exhibit changes in either the plasma inorganic phosphate ooncentration or the daily urinary inorganic phosphate content. The possibility of a relationship between renal sodium and inorganic phosphate excretion is discussed. Probable sources of the excess urinary inorganic phosphate are also discussed.

During the period of sodium bicarbonate ingestion two ponies exhibited decreased plasma urea concentrations, but no changes in urinary urea excretion were detected. The reason for another pony excreting abnormally large quantities of ammonium is unknown.

GENERAL CONCLUSIONS

Before comparing data obtained on the ponies studied in this thesis with that on other equines and on other species, it is essential to assess how typical the five ponies were of the Shetland breed, and, furthermore, how closely this breed is "typically" equine. A paucity of work on Shetland ponies made such an assessment difficult, and although studies of Shetland ponies [1, 17] have been reported, these, in common with the work herein, almost certainly would have involved the animals' removal from the natural island environment with its harsh climatic conditions and often poor "sea-drenched" grazing. Hence, Shetland ponies which are permanently housed, unexercised and regularly provided with adequate food merit but limited comparison with those in their natural state. Therefore, any characteristics which confer a special ability to survive adversity may be less manifest under experimental conditions unless these characteristics are inherited. Experimental work with Shetland ponies imposes therefore this limitation.

Time and facilities did not permit investigation of other Shetland ponies, neither was it possible to study the present ponies under different management conditions. Had this been possible, an insight might have been gained into physiological and biochemical changes brought about by housing, the provision of fodder, and a temperate climate.

The packed cell volume percentages of these ponies were similar to those reported by other workers who studied the breed [1, 17] and conformed with the general pattern illustrated in Table No. 1, where it is evident that packed cell volume percentages of thoroughbreds are generally higher [4,9,14] than those of slower moving draught horses and ponies [1,9,13,17]. It is widely accepted that the horse is adapted for a "fright and flight"

defence mechanism, but it was beyond the scope of this study to examine the performance of the ponies in this respect. The significance of changes in venous packed cell volume when the plasma and "thiocyanate space" volume is changed will be referred to later, when the implications of the results listed in Section No. 3 are discussed.

Doubtlessly the most remarkable discoveries made in this work relate to the ponies' ability to regulate plasma sodium levels. In man [53] and dog [193] the plasma sodium levels in health fall within fairly narrow limits, whereas comparatively large fluctuations occurred normally within and between ponies. Workers [1,3,16] who studied other types of equines discovered narrower ranges of plasma sodium concentrations than these observed in Shetland ponies. Alexander [81] reported day-to-day fluctuations in the plasma sodium concentrations which were approximately seven times greater in his Shetland and Shetland-cross ponies than in a group of hunters. Unfortunately the day-to-day variations within individual equines could be concealed by the way data is presented by authors, see Table No. 2.

With one exception [23] the mean plasma sodium concentrations of the ponies were lower than those of other equines in the literature reviewed. Only Alexander [23] reported a lower mean value, in his permanently housed, unexercised Shetland and Shetland-cross ponies. Thus, it appeared that plasma sodium levels of Shetland ponies differ from those of other equines both in being on average lower and in the extent to which they fluctuate about this mean.

Because of evidence which suggested that age [80], diet, and lack of exercise [82] alone were not likely to be responsible for the low plasma

Ref 193 Robinson, F.R., Zeigler, R.F., (1968) Lab. Animal Care 18 39.

sodium values observed, the question arises whether a low and variable plasma sodium concentration is a genetic feature of the Shetland breed, and, if so, whether the characteristic confers an advantage upon the pony.

Since the diet of a herbivore has a low sodium content compared with that of carnivores and omnivores they must conserve more ingested sodium. Tasker [86] and Alexander [23] both indicated that a highly efficient sodium conservation mechanism operates in equines. Further evidence of sodium conservation was provided by the pony (Scruffy), whose unilateral parotid duct fistula caused the sodium content of the saliva to be excreted rather than swallowed with the food. Despite ingesting more sodium his urinary and faecal sodium loss, both in terms of actual quantity and loss/kg body weight/day was less than that of the other four normal ponies, thus indicating his urinary and faecal sodium excretion was reduced to compensate for the loss of sodium in his saliva and thereby to maintain his plasma sodium concentration. It has also been observed[194] that although salivary urinary and faecal sodium contents were further reduced when the sodium supplement normally given to this pony was witheld for two weeks, no significant decrease in his plasma sodium level was detected.

Since no report ofasimilar investigation is known it is debatable whether the Shetland pony has a greater ability than other equines to conserve sodium. However, when in its natural environment where sodium intake may fluctuate widely this ability to regulate sodium excretion is possibly vital.

Ref 194 Alexander, F. (1973) Personal communication.

When the ponies ingested ammonium chloride and sodium bicarbonate further evidence of their remarkable ability to regulate the plasma sodium concentration was apparent. In addition to inducing metabolic acidosis, a very pronounced natriuresis accompanied the ingestion of ammonium chloride. However, despite this natriuresis, hyponatraemia was never evident. Moreover, during ingestion of sodium bicarbonate, which imposed a heavy sodium load, hypernatraemia was not observed, though an even more pronounced natriuresis than that which accompanied ammonium chloride loading occurred.

Thus the ponies were obviously able to deal with both excessive and deficient dietary intakes of sodium. Whilst natriuresis and diuresis is associated with ammonium chloride ingestion by man,[53] natriuresis in the ponies occurred without a concurrent diuresis regardless of whether the source of sodium was endogenous or exogenous.

From the estimated sodium intake and the measured urinary losses it was deduced that during ammonium chloride-induced natriuresis, the ponies were in negative sodium balance. Furthermore the very reduced output of urinary sodium on cessation of ammonium chloride loading which ultimately returned to the previously established normal output, strongly suggests the sodium reserves were being replenished in the "post loading" period. In contrast, after sodium bicarbonate loading ceased, the enhanced urinary sodium excretion promptly returned to normal.

These observations raise an important question concerning the source of the excess urinary sodium during natriuresis. In view of Alexander's[23] discovery of large quantities of sodium in gut fluids, and the observation that the equine gut normally contains over 100 litres of water, much of which it is believed can be absorbed [88,93], it is feasible that sodium from gut fluid might be absorbed into the body and excreted in urine, thereby maintaining plasma sodium levels during natriuresis.

It was demonstrated [172] that the sodium content of canine tibial cortical bone decreased in dogs maintained for 5 to 10 days in a state of metabolic acidosis. If tibial cortical bone typifies bone from other sites, it is possible that the large skeletal mass of equines is a source of sodium in times of negative sodium balance. However, in view of the obvious experimental difficulties involved, no known attempts have been made to determine the source(s) of sodium in such times of need in equines, nor has the relative importance of various sources of sodium been established.

It is obvious both from the work described in this thesis and from reviewing relevant literature, that much work still needs to be carried out to answer the basic question raised concerning regulation of plasma sodium concentration in equines. Comparatively the amount of relevant information amassed in other species far exceeds that gained from equine studies. Whilst forseeably some studies would be difficult owing to the size of the equine, some aspects of the present work could forseeably be extended without undue difficulty. Thus a study of concurrent changes of sodium lost in faecal fluid and urine might reveal a decrease, if not a cessation, in faecal sodium excretion when renal excretion was high, in an attempt to conserve sodium. However, if the sodium content of the gut fluid did indeed act as a source of sodium for the pony, faecal fluid sodium content may not reflect entirely changes which could be occurring in more cranial regions of the gut, where sodium concentrations are greatest. Fistulated ponies would almost certainly be needed for such an investigation of the less accessible regions of the digestive tract.

In retrospect, the studies of the effect on the ponies of ammonium chloride and sodium bicarbonate loading could have been improved and

extended in the following ways, in addition to studying faecal fluid as described above.

First, it would have been advisable, before the major experimental work was commenced, to have administered a single dose of the acidifying and alkalosing agents, and monitor over the following few hours changes in acid/base parameters and in the plasma constituents. Thus, the time of the maximum effect of these salts upon acid/base balance and upon the plasma constituents relative to the time of their administration could have been ascertained. Hence, if blood sampling was undertaken then, instead of 24 hours after dosing, greater fluctuations in the plasma constituents might have been observed.

Second, the induction of metabolic changes by parenteral administration of acidifying and alkalosing agents might have eliminated any variation between ponies arising from differences in absorption of the salts from the gut. Although this could create practical difficulties over long periods, such difficulties may not be unsurmountable.

The plasma potassium concentrations of these ponies did not appear to differ markedly from those reported in other equines [3,4,14,22,24], but it appears that the equine differs from the human [53] insofar as many plasma concentrations observed and reported in apparently healthy equines [8] fell outwith the accepted normal concentration range in man of 3.5 to 5.5 mEq/L[53]. Since the herbivore's diet is potassium rich and their urinary excretion indicates elimination of a large excess, low plasma potassium concentrations reported in equines could not be readily attributed to a potassium-deficient diet. Furthermore, differences occurred between the response of the five experimental ponies and other species when the effects upon selected plasma constitutents of ammonium chloride and sodium bicarbonate were investigated. Thus, acidosis in man and in dogs is usually associated with hyperkalaemia[53,173,174,175,176,177], which in turn is commonly followed by enhanced urinary

potassium excretion. Conversely, hypokalaemia is associated with alkalosis in these species [53,176,177,182]. In dogs, so close is the correlation between blood pH and plasma potassium concentration that an inverse change of 3.0 to 5.0 mEq/L in the plasma potassium concentration accompanies a change of 1 pH unit in blood [177]. Despite the induction of a quite severe metabolic acidosis in the Shetland and Shetland-cross ponies no such correlation was manifest. Neither, though the sodium bicarbonate load given induced a trend towards but not a significant alkalosis, was there a concurrent fall in plasma potassium concentration. Since no other reference to an investigation of this type in equines was discovered, it is not known whether the absence of a change in plasma potassium concentration when the pH of blood shifts is a widely occurring phenomenon in equines, or whether it is peculiar to these ponies.

This finding obviously merits further consideration and an investigation of whether it is another example of a specific ability of a Shetland pony to regulate plasma constituents under adverse conditions. As would be expected, the urinary excretion of potassium appeared closely related to the ponies' hay intake, and while it was noticed that a decrease in urinary potassium occurred after the pony's refusal of part of the daily hay ration, no concurrent significant diurnal fluctuations occurred in the plasma potassium concentration.

In view of the known influence of the adreno-corticoids on sodium and potassium in other species, a study of these hormones in equines, with special emphasis on the regulation of sodium and potassium, might provide valuable information on the ability of these ponies to control their plasma electrolyte levels. Electrocardiographic examination of horses and ponies exhibiting markedly low plasma potassium concentrations might reveal similar effects to hypokalaemia in man and dogs though it appears the equine myocardium is less susceptible to low plasma potassium levels than the human heart.[53]

No gross difference between the plasma chloride concentration of these ponies and those of most equines [3,4,24,25], nor between equines and other domestic species [195,196,197,198], with the probable exception of the cat[199], was evident. However, in view of the reciprocity of the plasma chloride and bicarbonate concentrations in other species [53,178], a more detailed study of the relationship between these two plasma constituents in equines would be worthwhile, especially if an "alkaline tide" phenomenon[105] could be demonstrated.

Although these ponies exhibited plasma inorganic phosphate concentrations similar to those cited in horses [1,14,25,27] and in other ponies [1,23], the method of presentation of data by other workers gives no indication of whether day-to-day fluctuations in the plasma inorganic phosphate concentrations occurred in their individual animals.

It is known [50] that fermentation of cellulose is a vitally important biochemical process occurring in the rumen of cattle and sheep. Here the volatile fatty acids produced are buffered chiefly by bicarbonate, much of which is derived from the copious volume of saliva ruminants swallow with the food.

195 Michaelson, S.M., Scheer, K., Gilt, S.(1966) J.Am Vet.Med.Assoc.148 532.

196 McSherry, B.J., Grinyer, I. (1954) Am.J.Vet.Res. 15 509.

197 Pugh, S.M. (1966) Ir. vet.J. 20, 142.

198 Bertho, E.M., Belianger, M., Saint-Hilaire,B.(1964) Laval Med. 35, 647.

199 Spector, W.S.(1956) ed. "Handbook of Biological Data" Saunders, Philadelphia, U.S.A.

In the equine gut, cellulose digestion occurs in the large intestine[23], and although Alexander[23] discovered high bicarbonate concentrations in the ventral segment of the equine colon, he was able to demonstrate that there was a pronounced change in the dorsal regions of the large colon, where inorganic phosphate concentration rose, whilst that of bicarbonate fell. Of the plasma constituents studied in this work only inorganic phosphate was excreted in greater quantities in faecal fluid than in urine. This is consistent with the discovery by Alexander[23] of high concentrations of inorganic phosphate in the distal regions of the equine gut. Since Alexander[23] postulated the major role of this inorganic phosphate was to buffer volatile fatty acids produced by cellulose fermentation, a knowledge of why there is a change in the predominance of the bicarbonate and inorganic phosphate buffers in different regions of the equine large colon might provide a valuable insight into equine gut physiology.

Equine urine like that of sheep normally contains comparatively little inorganic phosphate. However, unlike sheep [98] the Shetland and Shetland-cross ponies responded to an acid load by a vast increase in urinary inorganic phosphate excretion, whilst showing only a small increase in the urinary ammonium content.

In man and dog increases have been observed in urinary phosphate excretion during metabolic acidosis and these species have been discovered to be in negative phosphate balance if the phosphaturia became excessive[183]. However, the plasma inorganic phosphate levels of these ponies when acid loaded remained within normal levels despite an increased urinary phosphate excretion. Since neither phosphate intake nor faecal phosphate losses were studied during ammonium chloride ingestion, it is not known whether the ponies were in fact in negative phosphate balance at this time.

Roemmelt and Pitts[179] deduced that in man the source of the excessive urinary phosphate was intracellular. However, in view of the high concentrations of inorganic phosphate in the large volumes of equine gut[23] fluid it is possible some of this phosphate is available for absorption and renal excretion when required. The reason for the predominance of the phosphate buffering system over the ammonium buffering system under conditions of metabolic acidosis, and the source of the excess inorganic phosphate excreted in urine are worthy of further study.

The administration of a constant ammonium chloride load to rats results in a progressive increase in renal glutaminase activity which is closely paralleled by an increase in ammonium excretion[184]. No reference to the level of glutaminase in equine kidneys was discovered, but if this was low, and a source of inorganic phosphate was readily available to the animal, it seems logical that phosphate excretion could take precedence over ammonia formation, and act as the major urinary buffer.

It was discovered in dogs[168] that the tubular reabsorption of sodium influenced that of inorganic phosphate such that an increase in the former was associated with a similar increase in the latter. Since phosphaturia accompanied the natriuresis observed over the time the ponies ingested ammonium chloride or sodium bicarbonate, it appears not unlikely that urinary sodium excretion in ponies influences the renal excretion of inorganic phosphate. However, it was concluded that as close a relationship between excretion of ions does not exist in ponies as in dogs.

Unlike carnivores and omnivores, but in common with other equines, excluding racehorses immediately after exercise[91], the pH of the urine of these ponies in the untreated state was alkaline, and, almost without exception, net base was excreted. It was evident that the measurement of the daily excretion of net acid/base by the kidney was a better index of

the acid/base status of the animal than urine pH; furthermore net acid could be excreted when urine pH was alkaline, and, conversely, net base could be excreted when the urinary pH was below 7.40. The venous blood pH of these ponies was frequently below the often-quoted average human venous blood pH of 7.42, but owing to a paucity of published data on venous blood pH in other equines, comparisons with the pH values of the blood samples from the Shetland ponies were of limited value.

The reasons for an attempt being made to measure plasma and thiocyanate space volumes of the ponies in the untreated state and again immediately before and after the administration of a water load have been described in the General Introduction, and in the Introduction to Section No.3. However, the results obtained from these experiments were not quite as definitive as one might have wished and their interpretation presented considerable difficulty, as has been already discussed.

All measurements of body fluid compartment volumes are subject to errors arising from the assumptions that the substance whose dilution volume is measured fulfils the basic requirements described [116] and that during the period of measurement, the body fluid compartment volume remains unchanged [117,124]. However, though this widely accepted dilution technique was exploited in an attempt to measure dynamic changes in body fluid compartments, it is believed that in fact additional sources of inherent error were introduced because of the experimental protocol, thereby complicating an interpretation of the results obtained. Because T-1824 binds closely with plasma albumin [134,139] it was concluded that the accuracy with which T-1824 reflected changes in plasma volume was likely to be greater than that which sodium thiocyanate reflected changes in a body fluid volume which, though possibly not strictly identical to the volume of extracellular fluid, is believed approximates closely to it [154] in some other species.

One major disadvantage of the thiocyanate ion is its ability to diffuse not only throughout the plasma and interstitial fluid, but also into ocular, cerebro-spinal, glandular and, of particular significance, into digestive tract fluids [116].

Furthermore, because the concentration of the ion in these fluids is probably not identical with the concentration in interstitial fluid, which, in turn may not be identical with the concentration in the fluid which is actually sampled - namely plasma - the apparent volume of distribution of the ion may not reflect accurately any well-defined body fluid compartment[150]. Strictly, the fluids within the digestive tract are outwith the body. Hence in the ruminant, and in equines, where a very large quantity of fluid is contained within the gut c.f. carnivores and man, this problem assumes even greater importance, and the ideal substance for such investigations in all mammals would be one which, in addition to complying with Crandall and Anderson's criteria [116], diffused rapidly and evenly throughout the extracellular space without entering gut fluid. Unfortunately no such substance is known [148], and the measurement of total body water in addition to the thiocyanate space volume would be susceptible to the same errors as thiocyanate space determinations.

Nevertheless, the T-1824 and thiocyanate clearance curves clearly indicated an increase in the volume of the plasma and thiocyanate spaces following water loading. Whilst it was not a difficult mathematical exercise to define and construct the two components of the clearance curves, the interpretation of the graphs presented difficulties, the major one being the understanding of the significance of the inflection point, since it was believed unlikely that the absorption of the water load, or part thereof, would be sudden in onset, and of short duration.

Overhydration in cattle was observed to cause an initial haemoconcentration followed by haemodilution[156], and shifts in the body fluids of donkeys have been seen to follow a pattern whereby changes in circulating blood volume were minimised at the expense of thiocyanate space fluid and intracellular fluid [115].

It is appreciated that the corpuscular and plasma components of circulating blood are subject to different regulatory mechanisms, and since the venous haematocrit is unlikely to be identical with the "total body haematocrit" [9,17,18,20] it was not anticipated that any changes observed in the jugular venous haematocrit would accurately reflect changes in plasma volume, and it was also believed that in the event of plasma dilution, compensatory changes which minimised the potential decrease in plasma electrolyte concentrations would be evoked. Since plasma urea concentration had been shown to be affected by feeding and because urea diffuses throughout the body and gut fluids, falls in its plasma concentration would not be a reliable guide to plasma dilution either. From the nature of the diuretic response after water loading it was considered impossible to deduce either the time of onset or the magnitude of changes in T1824 and thiocyanate space volumes.

It was thus concluded, that although a great deal more work is required before these particular results can be meaningfully interpreted, the study nevertheless had been worthwhile, even if it only illustrated the difficulties and pitfalls of trying to extend an experimental method beyond the limits it was originally designed to attain and in a species it had not originally been devised for. Although, in view of the clinical implications of changes in body fluid compartments reliable measurement of such changes would be invaluable, the present work indicates the need to carefully assess the extent of "normal" variation within and between "normal" individuals before proceeding to investigate the abnormal.

In conclusion it seems these Shetland and Shetland-cross ponies clearly possess an astounding ability, apparently unparalleled by other species investigated, to thrive despite wide fluctuations in the concentrations of the plasma constituents studied. Moreover, they were also almost invariably able to maintain these plasma constituents within their particular normal concentration ranges when subjected to treatments which, in other species induce severe disturbances in acid/base balance and in plasma constituent concentrations. Of the plasma constituents studied the maintenance of the ponies' usual plasma sodium levels, despite water loading, the severe natriuresis which accompanied ammonium chloride-induced metabolic acidosis, and the heavy sodium load imposed by the daily ingestion of 3.5 to 4.6 mEq sodium/kg body weight, were especially remarkable. It is believed therefore that the ability of the Shetland pony to regulate its plasma constituents despite such severe challenges is a major contributory factor to the hardiness of the breed.

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APPENDIX No.1

INDIVIDUAL RESULTS OF ANALYSES OF BLOOD AND PLASMA FROM ALL PONIES

(i) PACKED CELL VOLUMES (%)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	33.5	33.5	30.0	41.5	29.0
	33.0	32.0	28.5	37.0	28.0
	34.5	32.0	32.0	43.0	29.0
	34.0	25.5	32.0	40.5	29.0
	33.5	26.5	34.0	37.5	29.5
	35.5	37.5	32.0	38.0	30.5
	36.5	37.0	34.5	35.0	34.0
	37.0	34.0	36.5	35.0	33.0
	35.5	32.5	34.5	26.0	33.5
	34.5	36.0	35.0	33.0	32.5
	36.0	35.0	35.0	40.5	23.5
	37.0	34.0	33.5	41.5	32.0
		33.5	35.0	37.0	31.0
		37.0	35.0	37.0	32.0
		36.5	28.0		

(ii) PLASMA SODIUM CONCENTRATIONS (mEq/l)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	128	130	134	132	133
	133	134	132	134	131
	133	135	133	123	131
	130	133	129	120	130
	133	128	133	131	133
	135	134	130	128	133
	136	138	135	123	140
	138	135	134	130	133
	138	135	138	135	138
	139	138	136	128	134
	125	137	133	135	138
	138	138	136	135	138
	129	131	130	138	139
		129	134	130	136
		136	132		
			131		

(iii) PLASMA POTASSIUM CONCENTRATIONS (mEq/l)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	3.10	5.00	3.70	4.20	3.75
	3.20	5.00	3.85	3.80	3.80
	4.40	3.80	4.00	2.70	3.85
	3.30	4.50	3.20	2.70	4.10
	3.50	4.90	3.80	3.80	4.20
	3.85	4.65	3.70	4.30	3.50
	4.60	4.35	3.70	4.00	3.70
	4.95	5.50	3.00	3.70	3.90
	4.20	5.40	4.05	3.80	3.95
	4.60	4.40	3.70	4.20	3.55
	4.30	4.80	4.40	3.40	3.70
	4.10	4.70	4.20	4.20	3.70
	4.75	4.50	4.15	3.90	4.00
		4.60	4.25	3.50	3.80
		5.10	3.90		
			4.10		

(iv) PLASMA CHLORIDE CONCENTRATIONS (mEq/l)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	99	102	102	105	97
	99	103	100	99	98
	101	102	98	98	101
	102	93	99	101	98
	103	104	97	98	98
	101	99	98	96	101
	106	102	102	102	103
	100	102	103	97	102
	101	98	104	99	105
	99	100	99	97	104
	100	101	101	95	98
	102	101	100	100	99
		101	101	98	98
		98	100	100	100
		99			

(v) PLASMA INORGANIC PHOSPHATE CONCENTRATIONS (mgP/100 ml)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	2.63	2.20	2.99	3.49	2.67
	3.03	3.61	3.47	3.13	2.08
	4.04	3.08	2.80	2.97	2.97
	3.39	3.08	2.72	3.34	2.71
	4.77	3.23	2.44	3.38	2.63
	2.42	3.89	2.67	4.16	2.49
	2.86	3.64	2.08	4.38	1.30
	3.95	3.61	3.34	3.89	2.44
	2.52	2.87	3.26	3.94	2.50
	1.28	3.20	4.30	3.29	1.76
	3.62	3.39	3.44	4.36	4.11
	2.58	2.48	3.23	4.21	2.77
		3.98	3.58	2.70	1.57
		3.40	2.93	2.79	2.48
		3.32			
		1.96			

(vi) PLASMA UREA CONCENTRATION (mg urea/100 ml)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	25.6	53.8	47.4	26.9	40.5
	30.9	67.3	46.1	31.9	31.0
	29.9	64.7	52.6	23.3	25.8
	47.9	58.3	27.6	42.3	23.8
	38.8	87.0	59.2	40.6	24.0
	31.9	65.0	57.6	29.6	27.5
	26.0	67.2	24.4	32.1	23.1
	20.0	53.1	29.1	49.4	27.4
	24.7	46.5	28.8	30.0	29.8
	27.0	49.2	22.6	51.0	31.5
	20.4	59.6	22.3	22.0	29.0
	26.2	48.2	23.7	17.7	20.3
		46.5	15.5	21.6	22.3
		55.5		28.9	
		42.2			

(vii) SPECIFIC GRAVITY OF WHOLE BLOOD

Pony	Scruffy		Jimmie		Billie		Ben		MacGowan	
	S.G.	PCV%	S.G.	PCV%	S.G.	PCV%	S.G.	PCV%	S.G.	PCV%
	1.047	31.5	1.054	35.0	1.069	31.0	1.060	41.0	1.058	27.5
	1.044	35.5	1.053	33.0	1.050	33.0	1.058	40.5	1.037	25.5
	1.056	36.0	1.049	31.5	1.050	31.5	1.060	39.0	1.043	25.5
	1.048	32.0	1.046	32.0	1.068	34.5	1.056	39.0	1.055	26.0
	1.048	33.0	1.054	35.0	1.056	37.5	1.055	39.5	1.046	25.5
	1.050	37.5	1.048	33.5	1.054	36.5	1.047	36.5	1.047	27.5
	1.052	34.0	1.053	36.5	1.054	35.5	1.056	39.5	1.046	25.5
	1.051	36.5	1.046	34.0	1.050	32.0	1.052	39.0	1.046	24.0

(viii) SPECIFIC GRAVITY OF PLASMA

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	1.025	1.024	1.027	1.029	1.031
	1.024	1.025	1.027	1.028	1.028
	1.025	1.028	1.031	1.024	1.029
	1.028	1.028	1.029	1.024	1.032
	1.025	1.027	1.030	1.025	1.026
	1.026	1.021	1.024	1.017	1.023
	1.029	1.030	1.025	1.026	1.025
	1.024	1.025	1.023	1.022	1.025

(ix) WHOLE BLOOD pH

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	7.395	7.390	7.385	7.395	7.375
	7.395	7.380	7.375	7.410	7.385
	7.410	7.410	7.390	7.360	7.385
	7.385	7.370	7.410	7.390	7.370
	7.395	7.370	7.280	7.380	7.420
	7.380	7.385	7.365	7.375	7.385
	7.410	7.410	7.365	7.395	7.420
	7.435	7.380	7.420	7.470	7.405
	7.420	7.430	7.365	7.440	7.390
	7.420	7.420	7.410	7.415	7.380
	7.430	7.390	7.410	7.410	7.395
	7.400	7.395	7.405	7.410	7.440
	7.410	7.425			
	7.385	7.400			
		7.430			

(x) WHOLE BLOOD pCO_2 (mm Hg)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	44.5	44.5	43.0	41.0	48.5
	47.5	44.0	45.0	43.0	42.5
	42.5	47.5	45.0	45.0	47.0
	38.0	50.0	42.0	45.0	46.0
	40.0	47.0	47.0	46.0	45.0
	48.5	48.0	48.0	44.0	49.0
	44.0	47.0	49.0	44.0	47.5
	50.5	51.0	47.0	40.0	47.5
	43.0	45.0	50.0	44.5	38.0
	46.0	45.0	46.0	45.0	51.0
	47.5	46.0	45.0	49.0	49.5
	46.5	45.0	44.0	48.0	44.0
	43.5	42.5			
	43.0	43.0			
		47.0			

(xi) SERUM BICARBONATE CONCENTRATIONS (mEq/l)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	29.7	28.1	28.0	28.2	27.5
	30.2	27.2	28.0	26.9	27.7
	29.8	29.2	26.2	24.5	27.3
	29.1	28.6	27.9	25.8	26.4
	29.5	27.4	25.7	26.4	28.3
	25.9	27.8	26.4	25.0	28.5
	28.0	28.8	27.0	26.0	29.6
	32.8	29.1	29.5	28.3	28.5
	26.9	28.7	27.5	29.3	30.5
	29.3	28.4	28.3	29.2	28.9
	30.5	29.4	27.6	29.9	29.2
	27.9	25.9	27.5	29.5	26.4
	26.7	27.1	28.8	23.7	
	25.9	25.6	28.8	24.9	
		29.4	28.0	25.7	
		27.6	28.6		
		27.0	28.9		
		29.5			
		27.8			
		26.3			

APPENDIX No.2

INDIVIDUAL RESULTS OF ANALYSES OF URINE FROM ALL PONIES

(i) VOLUME PASSED/24 HOURS (measured to the nearest 10 ml).

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	6500	4230	2970	2450	2000
	7650	4380	2980	1740	4950
	2810	3600	3090	1900	3440
	1920	2940	1940	2790	4780
	5650	3050	1990	1600	5440
	3310	2980	4270	2540	7820
	2880	3510	2310	1970	4610
	3610	3430	6390	3290	2860
	6350	1560	3720	3060	5230
	4080	6640	2700	1540	4580
	4560	3280	4140	2790	3010
	7840	1790	3900	2800	6600
	4170	1550	5080	4030	4520
	6790	4040	6180	5200	4700
	5630	2370	4330	2260	4000
	6860	2090	6430	2770	4060
	4670	3850	6530	5150	3610
	6250	1640	5650	4960	
	6390	3480	2750	5650	
	4700	2750	4350	5000	
	8200	2910	1690	4440	
	5610	3900	2240	4320	
	5080		3640	6390	
	8760			1640	
	5090			3170	

(ii) SPECIFIC GRAVITY OF URINE

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	1.025	1.027	1.044	1.045	1.045
	1.021	1.026	1.040	1.043	1.037
	1.036	1.030	1.031	1.041	1.041
	1.034	1.029	1.041	1.038	1.042
	1.015	1.028	1.037	1.049	1.039
	1.024	1.034	1.021	1.035	1.026
	1.030	1.025	1.030	1.048	1.025
	1.021	1.041	1.014	1.038	1.022
	1.014	1.025	1.030	1.030	1.021
	1.028	1.034	1.026	1.040	1.018
	1.016	1.031	1.023	1.039	1.022
	1.005	1.036	1.026	1.031	1.017
	1.024	1.024	1.021	1.023	1.023
	1.019	1.033	1.019	1.025	1.026
	1.012	1.033	1.023	1.026	1.020
	1.015	1.023	1.017	1.028	1.035
	1.015	1.030	1.017	1.019	1.027
	1.010	1.030	1.019	1.021	
	1.010	1.028	1.031	1.030	
	1.044	1.034	1.018	1.036	
	1.017	1.030	1.040	1.019	
	1.010		1.032	1.026	
	1.016		1.023	1.013	
	1.019			1.041	
	1.009			1.030	
	1.017				

(iii) pH OF URINE

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	7.35	8.80	8.95	8.75	8.65
	7.55	8.70	8.95	8.85	7.45
	8.15	8.90	9.00	8.55	8.55
	8.50	8.40	8.60	9.00	7.90
	8.65	9.10	8.60	9.05	8.05
	9.10	8.90	7.85	9.05	8.05
	9.20	8.10	8.35	8.55	7.80
	7.40	7.70	8.95	8.65	8.50
	8.05	7.70	8.30	8.95	8.95
	8.70	8.50	8.10	8.75	8.75
	8.65	7.85	8.35	8.50	8.95
	8.70	8.05	8.95	8.25	9.05
	8.90	8.70	8.40	8.50	8.95
	7.60	8.80	8.05	8.65	8.40
	8.55	8.50	8.40	8.95	9.05
	8.85	9.00	8.80	8.70	9.00
	8.90	8.65	9.00	8.70	9.10
	8.45	8.15	8.75	8.90	
	8.05	9.10	8.60	7.50	
	8.40	8.95	8.25	7.65	
	9.10	8.40	8.90	8.45	
	7.75	8.60	7.95	8.75	
	8.20		8.95	8.60	
	7.60			8.80	
	7.80			8.25	
	8.40				

(iv) NET ACID/BASE IN URINE (mEq/24 hours).

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	1022	417	430	775	250
	219	386	344	239	420
	-92	282	250	298	827
	649	262	354	434	1396
	311	303	407	512	813
	134	181	376	466	482
	348	284	442	649	352
	-102	456	192	476	493
	11	203	384	351	306
	75	456	421	491	274
	358	299	468		470
	420	253	129		258
	275	591	294		28
	343		484		30
	404				477
	428				133
	359				

NET ACID EXCRETION EXPRESSED AS -IVE

" BASE " " " +IVE

(v) SODIUM CONTENT OF URINE (mEq/24 hours).

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	81	173	178	100	103
	190	210	98	61	353
	28	83	148	20	327
	22	103	58	52	100
	37	153	70	24	90
	25	45	226	92	375
	26	102	127	49	46
	22	35	128	158	27
	184	109	130	285	52
	510	329	68	123	86
	23	41	62	120	60
	39	47	171	50	157
	250	16	114	151	90
	47	182	131	338	59
	35	65	70	145	150
	94	73	88	29	183
	35	231	141	206	129
	10	21	85	167	
	8	122		224	
	56	186		150	
	39				

(vi) POTASSIUM CONTENT OF URINE (mEq/24 hours).

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	1855	1350	1426	1271	880
	1800	1490	1788	757	693
	1349	1440	1020	912	1376
	691	1117	815	1172	2294
	1074	1007	716	880	2395
	794	1311	897	864	2424
	730	793	554	1044	1083
	816	1201	927	1530	529
	1016	515	1190	765	1046
	898	1760	581	554	870
	730	1115	1076	1228	617
	314	537	1073	1092	1089
	709	605	1143	927	1220
	1358	1010	1269	1118	1175
	845	265	953	429	960
	1029	752	1543	636	1279
	630	924	1371	1082	830
	781	558	961	992	
	1486	1670		1840	
	1438	935		2100	
	823				

(vii) CHLORIDE CONTENT OF URINE (mEq/24 hours).

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	1268	694	707	658	484
	1160	740	679	369	1238
	525	565	633	766	815
	276	368	326	491	1539
	548	482	237	320	1104
	391	614	675	363	1439
	311	611	434	514	516
	444	916	626	707	243
	699	368	662	428	246
	277	611	362	313	637
	274	624	596	622	394
	251	304	671	647	772
	125	327	701	451	687
	951	707	952	603	1086
	507	633	701	235	500
	700	472	868	391	881
	537	547	771	582	570
	588	282	571	630	
	501	623		1540	
	575	534		1475	
	343				

(viii) INORGANIC PHOSPHATE CONTENT OF URINE (mg P/24 hours).

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	70.3	111.5	65.4	261.4	3.2
	80.2	146.2	45.8	313.4	31.0
	42.7	63.1	26.0	197.8	4.5
	27.7	31.0	24.4	328.5	127.1
	82.0	56.8	35.6	89.4	77.0
	84.5	54.4	61.4	164.3	55.2
	96.5	147.2	74.1	259.7	17.9
	52.5	11.6	53.2	438.6	206.9
	83.8	80.8	157.4	154.2	89.6
	26.2	430.0	78.3	233.9	59.2
	9.3	129.6	107.4	314.8	82.6
	41.5	165.0	49.9	276.9	89.5
	109.0	166.0	76.4	111.8	93.5
	61.4	115.9	122.4	920.9	64.8
	60.4	61.5	76.6	481.3	105.2
	159.2	39.8	140.8	543.0	54.2
	54.7	76.6	86.5	236.8	
	76.9	62.2	27.5	382.8	
	63.0	105.8		236.4	
	22.9	97.1		578.5	
	9.4	643.1		190.9	
	39.0	457.5			
	67.6				
	83.8				
	45.6				
	52.8				

(ix) AMMONIUM CONTENT OF URINE (mEq/24 hours).

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	228	279	408	180	185
	219	281	323	263	144
	136	361	399	191	157
	155	119	107	255	47
	286	388	111	178	161
	331	273	131	135	85
	414	126	143	437	116
	85	188	411	218	420
	228	76	138	224	350
	98	108	36	176	403
	309	48	162	137	680
	189	0	399	157	597
	34	62	195	167	524
	115	278	102	230	614
	164	134	172	208	583
	359	289	465	380	
	514	74	581	86	
	205	20	394	224	
	114	577			
	167	234			
	436	164			
	173	150			
	223				
	187				
	168				
	267				

(x) UREA CONTENT OF URINE (g/24 hours).

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	28.8	28.2	25.6	52.5	30.2
	20.0	16.9	43.1	37.6	36.8
	20.8	12.9	27.5	24.4	71.2
	13.9	15.3	41.6	20.8	75.4
	13.0	14.4	45.0	25.9	82.0
	30.0	30.5	25.4	36.3	60.3
	21.8	35.2	32.8	24.7	43.6
	46.7	41.4	12.4	23.3	41.5
	22.1	33.4	38.8	31.6	22.7
	17.0	47.3	36.2	18.3	15.8
	37.9	70.5	23.9	27.9	36.8
	31.9	30.8	67.5	63.5	29.6
	27.5	26.3	34.4	20.0	35.8
	19.0	34.6	33.8	18.9	15.9
	18.5	33.0	39.9	24.0	39.6
	17.2	18.2	19.3	17.3	
	26.8	41.1	20.2	82.2	
	25.8	23.2	23.2		
	10.7	26.7			
		23.2			

(xi) * "TOTAL NITROGEN" CONTENT OF URINE (g/24 hours).

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	14.2	13.6	17.7	27.0	16.7
	11.5	8.3	25.8	16.1	19.2
	13.7	6.5	18.4	10.9	35.5
	11.4	10.6	20.9	21.3	36.0
	11.9	15.5	22.6	14.0	40.6
	34.9	18.1	13.7	13.3	29.4
	15.6	18.8	17.4	14.6	20.1
	23.2	22.0	11.6	18.8	25.8
	14.7	16.7	20.1	17.7	15.5
	10.5	23.6	17.4	13.9	13.0
	19.7	33.7	13.4	17.9	26.7
	16.5	14.4	37.1	13.5	22.2
	15.2	13.2	19.2	14.9	24.0
	13.9	20.0	17.2	31.9	16.0
	15.8	17.4	21.1	11.7	26.7
	10.9	12.6	15.4	12.0	21.7
	14.1	19.9	17.6	14.0	
	14.4	11.1	16.4	13.4	
	11.1	20.3		40.6	
		14.1			

* THE NITROGEN FROM AMMONIUM AND UREA ONLY

(xii) NET ACID/BASE CONCENTRATION IN URINE (mEq/l)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	250	134	67	149	51
	48	118	92	106	122
	-12	158	93	108	173
	156	169	86	84	257
	46	75	104	103	104
	24	76	74	105	105
	51	136	71	150	123
	-22	118	44	74	94
	2	124	60	214	67
	12	131	64	155	91
	56	109	36		71
	89	87	170		57
	33	152	30		6
	61		131		8
	80		133		117
	49				37
	71				

NET ACID EXCRETION EXPRESSED AS -ive

" BASE " " " +ive

(xiii) SODIUM CONCENTRATION IN URINE (mEq/l)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	10	41	60	41	52
	11	48	33	35	71
	7	23	50	11	95
	8	35	30	19	21
	9	50	35	15	17
	6	15	53	40	48
	30	29	55	25	10
	125	10	20	48	9
	5	70	35	93	10
	5	50	25	80	19
	60	13	15	43	20
	7	26	44	18	24
	6	10	22	37	20
	14	45	21	65	13
	7	27	16	64	38
	2	35	14	10	45
	1	60	22	40	36
	9	13	15	33	
	8	35		40	
		68		30	

(xiv) POTASSIUM CONCENTRATION IN URINE (mEq/l)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	285	320	480	520	440
	235	340	600	435	140
	480	400	330	480	400
	360	380	420	420	480
	190	330	360	550	440
	240	440	210	340	310
	255	225	240	530	235
	160	350	145	465	185
	220	330	320	250	200
	160	265	215	360	190
	40	340	260	440	205
	170	300	275	390	165
	200	390	225	230	270
	150	250	205	215	250
	150	110	220	190	240
	135	360	240	230	315
	125	240	210	210	230
	230	340	170	200	
	225	480		325	
	175	340		420	

(xv) CHLORIDE CONCENTRATION IN URINE (mEq/l)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	195	164	237	269	242
	152	169	228	212	250
	187	157	205	403	237
	143	125	168	176	322
	97	158	119	200	202
	118	206	158	147	184
	108	174	188	260	112
	123	267	98	215	85
	110	236	178	140	47
	68	92	134	203	139
	60	190	144	223	131
	32	170	172	231	117
	30	211	138	112	152
	140	175	154	116	231
	90	267	162	104	125
	102	226	135	141	217
	115	142	118	113	158
	94	172	101	127	
	78	179		273	
	90	194		295	
	73				

(xvi) INORGANIC PHOSPHATE CONCENTRATION IN URINE (mg P/l)

Pony	Scruffy	Jimmi	Billie	Ben	MacGowan
	10.8	26.4	22.2	106.6	1.6
	10.5	33.4	15.4	185.8	6.3
	15.2	17.5	8.4	104.9	1.3
	14.4	10.5	12.6	117.0	26.6
	14.5	18.6	17.9	56.7	14.2
	25.5	18.3	14.4	65.4	12.0
	33.5	41.9	32.1	131.8	6.3
	14.5	3.4	8.3	133.3	39.6
	13.2	51.8	42.3	50.4	19.6
	6.4	64.8	29.0	151.9	19.7
	2.0	39.5	25.9	112.8	12.5
	5.3	92.0	12.8	98.9	19.8
	26.1	107.0	15.0	27.7	19.9
	9.0	28.7	19.8	104.4	16.2
	10.7	26.0	17.7	104.8	25.9
	23.2	19.0	21.9	138.2	15.0
	11.7	19.9	13.3	45.9	
	12.3	37.9	4.9	116.6	
	9.8	30.4	15.4	163.0	
	3.6	35.3	24.0	229.5	
	2.0	211.1	28.3	96.3	
	4.7	117.3	17.8	59.8	
	12.0			14.1	
	16.5			114.3	
	5.2				
	10.4				

(xvii) AMMONIUM CONCENTRATION IN URINE (mEq/l)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	35	66	137	74	37
	29	64	108	94	42
	48	100	129	119	33
	81	40	55	100	9
	51	127	56	90	21
	100	92	31	41	18
	144	36	62	143	41
	24	55	64	142	80
	36	49	37	80	76
	24	16	13	63	134
	68	15	39	34	103
	24	0	102	30	132
	8	40	38	74	111
	17	69	17	83	154
	29	57	40	40	144
	52	138	72	77	
	110	19	89	15	
	33	12	70	45	
	18	166	63	45	
	26	85	145	32	
	93	56	85	96	
	21	38	90	54	
	42				
	37				
	19				
	52				

(xviii) UREA CONCENTRATION IN URINE (g/l)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	10.3	6.7	8.6	21.4	6.1
	10.4	3.9	14.5	13.5	10.7
	3.7	3.6	8.9	15.3	14.9
	4.2	5.2	21.4	8.2	13.9
	4.5	4.7	22.6	13.2	10.5
	8.3	10.2	6.0	11.0	13.1
	3.4	10.0	14.2	8.1	15.2
	11.5	12.1	1.9	15.1	7.9
	4.9	21.4	10.4	11.3	5.0
	2.2	7.1	13.4	6.5	5.3
	9.1	21.5	5.8	6.9	5.6
	4.7	17.2	17.3	12.2	6.6
	4.9	17.0	6.8	8.9	7.6
	2.8	8.6	5.5	8.3	4.0
	4.0	13.9	9.2	4.7	9.8
	2.8	8.7	3.0	3.5	
	4.2	10.7	3.1	14.6	
	4.0	14.2	4.1		
	2.3	7.7			
		8.4			

(xix) * "TOTAL NITROGEN" CONCENTRATION IN URINE (g/l)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	5.1	3.2	6.0	11.3	3.4
	6.0	1.9	8.7	9.3	5.6
	2.4	1.8	6.0	5.7	7.4
	3.4	3.6	10.8	7.6	6.6
	4.1	5.1	11.4	8.5	5.2
	9.7	6.1	3.2	5.2	6.4
	2.5	5.4	7.5	7.4	7.0
	5.7	6.4	1.8	5.7	4.9
	3.2	10.7	5.4	5.8	3.4
	1.3	3.4	6.4	9.0	4.3
	4.7	10.3	3.2	6.4	4.1
	2.4	8.0	9.5	4.8	4.9
	2.7	8.5	3.8	3.7	5.1
	2.0	5.0	2.8	6.1	4.0
	3.4	7.3	4.9	5.2	6.6
	1.7	6.0	2.4	4.3	6.0
	2.2	5.2	2.7	2.7	
	2.3	6.8	2.9	2.7	
	1.4	5.8		7.2	
		5.1			

* THE NITROGEN FROM AMMONIUM AND UREA ONLY

APPENDIX No.3

INDIVIDUAL RESULTS OF ANALYSES OF FAECES AND FAECAL FLUIDS FROM ALL PONIES

(i) WEIGHTS OF FAECES PASSED IN 24 HOURS (kg)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	4.380	4.185	4.040	5.620	6.420
	4.290	2.770	4.470	5.780	7.170
	2.730	2.210	4.550	5.105	5.270
	5.140	3.160	3.225	3.250	6.205
	3.670	5.075	5.210	4.230	5.130
	5.635	4.170	5.305	4.970	4.830

(ii) WEIGHT OF FAECES/kg BODY WEIGHT/24 HOURS (g)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	24.5	25.5	22.3	31.0	30.0
	24.0	16.9	24.6	31.9	33.5
	15.3	13.5	25.1	28.1	24.6
	28.8	19.2	17.8	17.9	29.0
	20.5	30.9	28.7	23.3	24.0
	31.5	25.4	29.2	27.4	22.6

(iii) % WATER CONTENT OF FAECES

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	79	78	80	79	79
	79	75	79	74	79
	80	72	83	81	80
	82	73	81	75	77
	80	77	80	81	79
	80	78	79	78	78

(iv) WATER CONTENT OF FAECES PASSED IN 24 HOURS (ml)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	3460	3260	3230	4440	5070
	3390	2090	3530	4280	5660
	2180	1590	3780	4140	4160
	4210	2310	2610	2440	4790
	2940	3910	4170	3430	4050
	4510	3250	4190	3880	3770

(v) WATER CONTENT OF FAECES/kg BODY WEIGHT (ml)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	19.4	19.9	17.8	24.5	23.7
	19.0	12.7	19.5	23.6	26.4
	12.2	9.7	20.8	22.8	19.4
	23.6	14.1	14.4	13.5	22.4
	16.5	23.8	23.0	18.9	18.9
	25.2	19.8	23.1	21.4	17.6

(vi) pH OF FAECAL FLUID

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	6.25	6.15	6.05	6.55	6.30
	6.55	6.50	5.95	6.30	6.30
	6.55	5.90	6.30	6.60	6.25
	7.15	5.85	6.15	6.45	6.35
	6.55	5.95	6.70	6.55	6.15

(vii) * NET ACID/BASE CONTENT OF FAECAL FLUID (mEq/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	-31	-11	-16	-14	18
	60	-18	-64	-33	0
	46	-41	-96	38	-29
	66	-75	-31	46	- 5
	15	170	- 7	9	64
	17	43	2	35	7

* NET ACID EXPRESSED AS -ive
" BASE " " +ive

(viii) SODIUM CONTENT OF FAECAL FLUID (mEq/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	41	82	93	64	44
	25	39	79	32	127
	10	44	118	23	39
	21	40	32	37	45
	15	39	34	114	40
	20	18	37	27	139

(ix) POTASSIUM CONTENT OF FAECAL FLUID (mEq/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	208	179	178	444	380
	220	89	194	278	340
	158	87	302	286	489
	400	167	196	177	347
	206	283	313	274	354
	383	236	398	281	236

(x) CHLORIDE CONTENT OF FAECAL FLUID (mEq/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	21	33	23	169	20
	34	8	21	17	45
	0	10	11	4	20
	39	0	8	5	15
	6	8	13	10	8
	18	7	17	16	19

(xi) AMMONIUM CONTENT OF FAECAL FLUID (mEq/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	28	45	39	102	26
	56	23	71	48	26
	6	5	69	13	65
	4	38	60	9	36
	8	97	81	33	100
	76	51	71	73	38

(xii) UREA CONTENT OF FAECAL FLUID (g/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	0.9	1.0	0.7	1.7	1.3
	0.3	0.4	1.4	0.8	0.1
	0.6	0.6	3.4	0.7	14.7
	0.5	2.6	1.7	0.0	1.9
	1.1	5.2	4.5	1.0	5.4
	5.7	3.4	0.9	6.1	3.0

(xiii) * "TOTAL NITROGEN" CONTENT OF FAECAL FLUID (g/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	0.8	1.1	0.9	2.2	1.0
	0.9	0.5	1.7	1.0	0.4
	0.4	0.3	2.6	0.5	7.8
	0.3	1.8	1.6	0.2	0.6
	0.6	3.7	3.2	0.9	3.9
	3.7	2.3	1.4	3.8	1.9

* THE NITROGEN FROM AMMONIA AND UREA ONLY

(xiv) INORGANIC PHOSPHATE CONTENT OF FAECAL FLUID (mg P/24 hours)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
	4279	1465	3556	3175	-
	1383	939	5161	1260	6400
	2310	2590	7714	3136	1373
	4940	1222	3346	3290	4131
	2896	5558	5090	2313	7721
	4978	2337	5905	5061	4681

APPENDIX No.4

THE EFFECT OF FEEDING UPON THE PACKED CELL VOLUME PERCENTAGE, AND THE CONCENTRATIONS IN PLASMA OF UREA AND SELECTED ELECTROLYTES.

Individual Results.

(i) WEIGHT OF HAY CONSUMED, MEASURED AT HOURLY INTERVALS (kg)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
Hours after feeding					
1	0.75	0.50	0.50	0.50	0.75
2	0.50	0.50	0.50	0.50	0.50
3	0.25	0.00	0.50	0.50	0.50
4	0.25	0.75	0.25	0.25	0.50
5	0.25	0.25	0.50	0.25	0.25
6	0.00	0.25	0.25	0.50	0.50
7	0.25	0.25	0.25	0.25	0.25
8	0.25	0.25	0.25	0.00	0.25
Total eaten over the 8 hr period	2.50	2.50	3.00	2.75	3.50

(ii) PACKED CELL VOLUME (%)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
Hours before feeding					
8	35.0	35.5	24.0	37.5	28.0
7	33.5	26.5	27.0	34.0	26.0
6	33.5	24.5	30.5	34.5	-
5	32.5	28.0	30.0	36.0	24.5
4	34.0	26.0	31.5	35.0	24.0
3	37.0	25.5	30.0	38.5	24.0
2	37.0	28.0	31.5	38.0	23.5
1	38.5	27.0	33.5	38.5	24.5
Feeding					
Hours after feeding					
1	34.5	36.5	35.0	38.5	35.5
2	37.0	37.5	36.5	38.5	36.5
3	38.0	38.5	36.5	39.0	37.0
4	36.3	34.5	38.5	39.5	37.0
5	37.0	34.0	37.5	38.5	35.5
6	37.5	35.5	38.0	40.0	36.0
7	37.0	34.5	37.0	39.0	36.5
8	36.0	35.5	37.0	40.0	33.5

(iii) PLASMA UREA CONCENTRATIONS (mg urea/100 ml)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
Hours before feeding					
8	15.8	65.0	18.9	28.7	40.5
7	16.4	64.3	19.4	28.7	43.7
6	16.6	66.7	20.0	27.3	43.9
5	17.5	68.6	19.7	26.7	44.6
4	19.2	68.6	22.5	27.6	51.8
3	19.1	71.5	23.6	31.7	43.7
2	20.1	73.5	24.6	29.6	46.6
1	17.4	69.6	26.7	26.8	46.5
Feeding					
Hours after feeding					
1	43.8	65.5	41.3	35.5	43.3
2	44.3	68.9	47.0	38.0	41.5
3	47.8	91.4	47.0	36.7	60.5
4	50.1	78.3	46.3	37.9	61.1
5	51.4	101.2	51.2	36.0	49.1
6	50.2	94.2	53.0	34.2	47.7
7	52.0	110.1	53.0	30.7	52.0
8	51.7	105.8	50.8	35.4	49.1

(iv) PLASMA SODIUM CONCENTRATIONS (mEq/l)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
Hours before feeding					
8	138	133	130	135	131
7	131	133	129	133	133
6	129	128	129	135	134
5	131	128	129	134	134
4	133	135	130	134	125
3	135	133	125	131	133
2	133	131	130	135	133
1	136	131	128	133	135
Feeding					
Hours after feeding					
1	138	139	135	137	135
2	135	135	133	136	135
3	133	138	143	135	133
4	133	133	136	133	135
5	135	135	134	133	135
6	140	131	135	135	130
7	140	133	133	135	135
8	139	134	135	135	138

(v) PLASMA POTASSIUM CONCENTRATIONS (mEq/l)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
Hours before feeding					
8	4.45	4.70	4.00	3.70	3.80
7	4.00	4.00	4.15	4.00	3.90
6	3.70	4.25	4.25	3.60	3.50
5	3.85	4.05	4.20	3.15	4.10
4	3.85	4.10	3.90	3.80	3.75
3	4.00	4.25	3.85	3.70	3.60
2	4.10	4.25	4.30	3.65	3.60
1	3.60	4.35	4.00	3.45	3.70
Feeding					
Hours after feeding					
1	3.50	4.20	3.60	3.40	4.00
2	3.80	4.90	4.10	4.40	3.70
3	4.10	5.05	3.40	3.90	4.30
4	4.70	4.95	4.10	4.15	4.10
5	4.50	4.30	4.10	4.40	3.90
6	4.50	5.20	3.40	3.80	3.00
7	3.10	4.60	3.40	3.10	3.00
8	3.50	5.05	3.90	3.30	4.30

(vi) PLASMA CHLORIDE CONCENTRATIONS (mEq/l)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
Hours before feeding					
8	103	99	106	99	98
7	103	98	109	102	102
6	101	100	110	101	100
5	106	101	107	96	102
4	105	97	109	100	101
3	107	99	106	99	100
2	112	97	106	99	99
1	109	99	108	99	100
Feeding					
Hours after feeding					
1	102	103	101	102	100
2	103	103	101	103	103
3	103	100	102	101	99
4	102	102	102	103	101
5	101	102	102	103	100
6	104	100	100	101	97
7	102	100	100	100	98
8	100	103	99	101	98

(vii) PLASMA INORGANIC PHOSPHATE CONCENTRATIONS (mg P/100 ml)

Pony	Scruffy	Jimmie	Billie	Ben	MacGowan
Hours before feeding					
8	-	3.61	3.96	3.18	2.08
7	5.22	2.48	3.57	3.55	1.45
6	4.92	2.50	3.34	3.32	2.52
5	4.89	2.61	3.85	3.36	2.06
4	5.08	2.30	3.41	3.45	1.97
3	4.87	2.30	3.60	4.18	2.18
2	4.83	4.18	3.75	-	2.92
1	4.68	2.26	4.10	3.55	2.62
Feeding					
Hours after feeding					
1	4.22	3.13	3.38	3.19	2.08
2	3.93	2.11	2.37	3.37	1.93
3	3.63	1.90	2.33	2.78	2.20
4	3.32	1.66	1.80	2.75	2.10
5	3.53	2.02	2.22	2.37	1.38
6	3.31	1.73	1.86	2.44	1.49
7	3.43	2.13	1.81	2.44	1.49
8	3.68	1.83	2.65	2.60	2.12